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Abstract

Otolith chemistry (Ba:Ca, Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca, $\delta^{13}C$, and $\delta^{18}O$) was analyzed with sector field-inductively coupled-mass spectrometry (SF-ICP-MS) and isotope ratio-mass spectrometry (IR-MS) for 432 age-0 red snapper belonging to the 2005, 2006, and 2007 yearclasses and sampled among 6 Gulf of Mexico (Gulf) nursery regions. Otolith chemical signatures were significantly different among regions and yearclasses (MANOVA, $p<0.001$). Yearclass-specific linear discriminant functions parameterized with otolith chemical constituents distinguished nursery regions with between 67.4 and 87.5% accuracy. Otolith chemical signatures then were used to parameterize maximum likelihood models to estimate nursery source(s) of sub-adult and adult red snapper (n = 1,123) that were sampled among Gulf regions in summer 2006-2008 and whose otoliths had been cored and analyzed with SF-ICP-MS and IR-MS. Results indicated a local nursery origin for small, young red snapper on the west Florida shelf, and that little mixing between red snapper populations in US and Mexican waters occurred. A greater amount of interpopulational mixing between the eastern and western Gulf was indicated than has been reported previously, but that result should be interpreted with caution given the lack of distinctiveness of otolith chemical signatures for age-0 fish sampled in north central versus northwestern Gulf regions. Alternatively, this result may indicate that the larger red snapper population in the northwestern Gulf is serving as a source region of recruits as the stock recovers from being severely overfished. Overall, study results indicate that otolith chemistry could serve as an effective tool to examine recruitment dynamics and stock expansion as the Gulf red snapper stock continues its recovery.

Executive Summary

Otolith chemistry has been shown to be an ideal natural tag of bony fish populations or nursery areas. This is due to the fact that the chemical composition of the water in which fish live is permanently stored in the structure of otoliths, and otoliths themselves are metabolically inert once formed. This property of otoliths has been utilized in studies of fish migratory patterns and population connectivity, as well as to estimate the contribution of potential nursery areas to adult populations. In the current study, the source of red snapper recruits to northern Gulf of Mexico regions was estimated based on natural tags first derived from otolith chemical signatures of age-0 fish and then applying those signatures to ones measured in sub-adult and adult fish. Chemical constituents examined included Ba:Ca, Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca, which were analyzed with sector field-inductively coupled plasma-mass spectrometry (SF-ICP-MS), and $\delta^{13}C$ and $\delta^{18}O$, which were analyzed with isotope ratio mass spectrometry (IR-MS).

A critical assumption of this study was that the age-0 portion of sub-adult and adult otolith cores could be assayed accurately. Therefore, a series of experiments first was conducted to test the effect of cutting, coring, and pulverizing red snapper otoliths on their chemical composition. Results indicate that there is no difference in otolith chemistry between right and left sided sagittae, and that clean conditions and methods prevent contamination of otolith chemical signatures. There was a slight systematic shift in both element:Ca ratios (Ba:Ca, Li:Ca, and Mn:Ca) and stable isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) between whole and cored age-0 otoliths that likely resulted from the primordium being disproportionately represented in the cored versus whole otoliths. However, computing residuals of constituent values was shown to be an effective means to control for this bias.

Age-0 red snapper (n = 432) were collected among 6 Gulf regions in fall 2005-2007. Chemical signatures were significantly different among regions and yearclasses (MANOVA, $p<0.001$). Mean region-specific classification accuracies computed from linear discriminant function models ranged from 67.4 to 87.5% among the three yearclasses, but dropped to 49.0% when all yearclasses were included in a single model. The difference in otolith chemical signatures between age-0 red and lane snappers also was examined to investigate whether lane snapper signatures could be utilized as a surrogate in regions where age-0 red snapper samples were difficult to obtain. However, substantial differences in chemical signatures between species indicated that approach would not be feasible.

Otolith chemical signatures of age-0 red snapper were used to parameterize maximum likelihood models to estimate nursery source(s) of sub-adult and adult red snapper (n = 1,123) sampled among Gulf regions in summer 2006-2008 and whose otoliths were cored and analyzed with SF-ICP-MS and IR-MS. Results indicated a local nursery origin for small, young red snapper on the west Florida shelf, and that little mixing between red snapper populations in US and Mexican waters occurred. A greater amount of interpopulational mixing between the eastern and western Gulf was indicated than has been reported previously, but that result should be interpreted with caution given the lack of distinctiveness of otolith chemical signatures for age-0 fish sampled in north central versus northwestern Gulf regions. Alternatively, this result may indicate that the larger red snapper population in the northwestern Gulf is serving as a source region of recruits as the stock recovers from being severely overfished. Overall, study results indicate that otolith chemistry could serve as an effective tool to examine recruitment dynamics and stock expansion as the Gulf red snapper stock continues its recovery.

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General Introduction

Red snapper, *Lutjanus campechanus*, have supported one of the more economically important fisheries in the US Gulf of Mexico (Gulf) for more than 150 years (Camber 1955; Carpenter 1965; Collins 1885; Goodyear 1995). When the commercial fishery began in earnest in the mid 1800s, it was centered on the west Florida shelf between Pensacola, Florida and south to Tampa (Stearns 1883). However, as early as the 1880s red snapper catches began to decline on the west Florida shelf and new fishing grounds were sought (Camber 1955). By the beginning of the 20th century, the Texas red snapper fishery had developed and was centered on the Galveston Lumps and landings from the west Florida shelf were being caught as far south as the Dry Tortugas (Camber 1955). The fishery also began to transition in the early 20th century from one operating near home ports to a more distant fishery as ice became available for preserving catches, schooners gave way to diesel-powered fishing vessels, and abundant red snapper were discovered on the Campeche Bank off Yucatan, Mexico. United States commercial landings peaked in the 1960s when a large percentage of the catch was coming from Mexican waters, but landings continued to decline after Mexican waters were closed to US fishermen in 1980 (Porch et al. 2007).

Fewer data exist to document the history of the recreational red snapper fishery in the Gulf. Camber (1955) reported a significant charterboat fleet targeted red snapper on the west Florida shelf following World War II, which was about the time of the creation of Alabama's artificial reef program and the start of the charterboat fishery out of Orange Beach, Alabama (Minton and Heath 1998). Florida landings constituted a large percentage of total recreational landings through the mid 1980s but dropped sharply until a recent increase since 1995, mostly due to landings in the Florida Panhandle (SEDAR 2009). Since the early 1990s, Alabama landings have represented nearly 40% of total recreational landings and have sparked a debate whether artificial reefs off Alabama have increased production of red snapper in the region, or if they serve as net sink to red snapper production (Szedlmayer and Shipp 1994; Cowan et al. 1999; Minton and Heath 1998; Shipp and Bortone 2009; Cowan et al. 2010).

Today, red snapper remain among the more targeted finfish species by both commercial and recreational fishers despite the Gulf stock being estimated to be severely overfished (Minton and Heath 1998; Stanley and Wilson 1990; Porch 2007; SEDAR 2009). After the peak in US Gulf landings in the mid 1960s at around 8 million pounds (Porch et al. 2007), a long, steady decline began in both catch and catch per unit effort (CPUE) to historic lows by the late 1980s (Goodyear 1992; Schirripa and Legault 1999). Declining catches, and the near commercial extirpation of red snapper from historically productive waters off west Florida, prompted management actions by the Gulf of Mexico Fishery Management Council (Gulf Council) aimed at rebuilding the Gulf red snapper stock (GMFMC 1981, 1989).

Federal assessment and management of Gulf red snapper is based on the assumption that fish in US waters constitute a single genetic stock (GMFMC 1981, 1989), but few data existed at the onset of intensive federal management to evaluate or test the unit stock hypothesis. Studies conducted in the1980s and 1990s to examine stock structure or population mixing in red snapper produced equivocal results. Authors of early population genetics studies generally reported no significant differences among regions (Johnson 1987; Camper et al. 1993; Gold et al. 1997; Gold and Richardson 1998; Heist and Gold 2000), but Bortone and Chapman (1995) did report differences in mitochondrial DNA allele frequencies existed among northern Gulf regions. More recent work on red snapper genetic frequencies, however, has provided evidence that red snapper

in the northern Gulf constitute a marine metapopulation (Pruett et al. 2005; Gold and Saillant 2007; Saillant et al. 2010). Results from most tagging studies also seem contrary to the hypothesis that northern Gulf red snapper constitutes a single, well-mixed stock in that most authors have reported that tagged red snapper demonstrated high site fidelity and moved little (Beaumariage 1969; Beaumariage and Bullock 1976; Fable 1980; Szedlmayer and Shipp 1994; Szedlmayer 1997). However, Patterson (2007) demonstrated that results from all red snapper tagging studies contain observations of some fish that were stayers (no net movement, thus indicating site fidelity) and some individuals that were movers, and that the scope of the study typically affected the proportions of stayers versus movers observed. Moreover, authors of several studies have reported movement of individual red snapper on the scale of 100s of km (Szedlmayer and Shipp 1994; Patterson et al. 2001c; Strelcheck et al. 2007; Addis et al. 2008).

Gulf red snapper continues to be managed as a single stock in US waters, but several lines of recent evidence caused a shift in the management paradigm for this stock in 2005. Since that time, the Gulf red snapper stock has been assessed as two distinct components, one east and one west of the Mississippi River, although stock status evaluations are estimated, and management regulations are applied, Gulf-wide, not regionally. The rationale for this shift is found in the results of collaborative studies conducted in the early 2000s that examined regional differences in Gulf red snapper population demographics, population genetics, and post-settlement movement. Results of population demographics studies revealed significant differences in growth rates and reproductive biology among Gulf regions (Cowan et al. 2003; Fischer et al. 2004; Jackson et al. 2007). Analysis of microsatellite DNA variance revealed no consistent differences in allele frequencies among regions, but estimates of variance effective population size (Ne_V) differed significantly among regions, with the population off Louisiana estimated to have a 10-fold greater genetic effective population size than populations off either Texas or Alabama. Red snapper from each of the regions examined thus were hypothesized to represent demographically different (genetic) populations, with the highest levels of successful recruitment and population productivity occurring on the shelf off Louisiana (Saillant and Gold 2006). Pruett et al. (2005) interpreted these results, as well as results from mitochondrial DNA analysis, as indicative of metapopulation structure existing among semi-isolated Gulf red snapper populations, and subsequent genetics work has corroborated that interpretation (Gold and Saillant 2007; Saillant et al. 2010). Lastly, results from otolith chemistry analyses indicated little mixing between the eastern and western Gulf but significant post-settlement movement of individuals between the northwestern and southwestern regions of the US Gulf (Cowan et al. 2003; Patterson 2007; Patterson et al. 2008). Those findings are consistent with tagging results in which adult red snapper tagged off Alabama and the western Florida Panhandle moved significant distances to the east and southeast, with several fish recaptured along the west Florida shelf between Cape San Blas and Tampa, but only one fish (out of 1,364 recaptures of 9,014 fish tagged among three studies) reported as being recaptured west of the Mississippi River outflow (Patterson et al. 2001c; Strelcheck et al. 2007; Addis et al. 2008).

Results of the combined studies cited above indicate metapopulation structure exists in red snapper among northern Gulf regions examined. This finding is significant because the central assumption of any management plan is that fish being managed constitute a single population or stock with near uniform life history traits (Gulland 1977; Ricker 1975). Knowledge of metapopulation structure and demography is critical to fishery stock assessment and management because separate populations within the fishery may possess unique life history traits resulting from genotypic differences or phenotypic plasticity (e.g., differences in

physiology, behavior, growth, fecundity, and/or disease resistance) (Sinclair et al. 1985; Stepien 1995). Thus, distinguishing management sub-units within a fishery is of paramount importance for both stock assessment and catch allocation (Hilborn 1985; Sinclair et al. 1985). Differences in life history characters among populations are thought to contribute at the metapopulation or species level to long-term adaptability, survival, and fitness. From a fisheries management perspective, they can also greatly affect estimates of stock productivity (Porch 2007; Secor 2002). Therefore, if red snapper demonstrate strong fidelity to a given region, thus weak connectivity among regions, then region-specific management approach would be indicated (Szedlmayer and Shipp 1994). Alternatively, if moderate site fidelity and connectivity are exhibited, then a metapopulation approach to stock assessment and management may be more appropriate (Thorrold et al. 2001). Lastly, if some populations or regions within a species' range tend to be significant sources versus sinks of recruits, then those populations or regions should be protected to ensure healthy populations across the species' range (Crowder et al. 2000).

Questions remain with respect to red snapper population structure and connectivity among regions that should be addressed such that the Gulf red snapper resource is managed most effectively. For example, as Gulf red snapper have begun to recover from overfishing, fishery-dependent data and anecdotal information suggests fish are more abundant now on the west Florida shelf than they have been in decades (Farren 2000; SEDAR 2009). However, it is unknown whether population expansion is being fueled by local recruitment or if other Gulf regions are providing recruitment subsidies to the west Florida shelf as conventional tagging data might suggest. It is also unknown how much connectivity exists between the red snapper population off south Texas and fish off northeast Mexico. Mexican landings and CPUE have decreased in recent years and the Mexican stock is estimated to be severely overfished (Garcia et al. 2002). If connectivity between Texas and Mexican waters is high, then it may be that Mexican fisheries serve as a sink for Texas recruits (Crowder et al. 2000).

The overall objective of this study was to examine red snapper population connectivity between the west Florida shelf and other Gulf regions, as well as to estimate the source(s) of recruits to waters off Texas, in particular the connectivity between Texas and Mexican waters. This was accomplished by developing natural, region-specific tags based on otolith chemical signatures of age-0 fish sampled from 6 regions of the Gulf (Fig. 1), and then applying those signatures to the core chemistry of sub-adult and adult red snapper to estimate region-specific sources of recruits. A complimentary study also was conducted to test assumptions about whether coring and pulverizing otoliths, which was necessary to extract the chemical signatures of sub-adult and adult fish, affected their chemical signatures. Background information specific to each of these study components is presented in separate report sections below, beginning with coring assumption testing, along with methods and results and discussion specific to each study component.

Otolith Coring Assumption Testing

Introduction

Accurate knowledge of population structure, including estimates of the source(s) of recruits to various regions within a species' range, is important for effective fisheries management (Crowder et al. 2000; Hamer et al. 2005; Secor et al. 2009). Otolith chemistry has been shown in recent years to be an effective natural tag of fish populations thus provides a tool for estimating recruitment and migration patterns among populations, hence population structure (Campana and Thorrold 2001; Elsdon and Gillanders 2003). Employing otolith chemical signatures as natural tags has been demonstrated to be effective in distinguishing juveniles from geographically distinct areas, and then in turn for estimating the contribution of different nursery areas to adult stocks (Thorrold et al. 1998, 2001; Rooker et al. 2001, 2003, 2008; Patterson et al. 2008). Otolith chemical signatures have been employed to examine recruitment dynamics and population connectivity in several marine and estuarine fishes (reviewed in Campana and Thorrold 2001; Elsdon et al. 2008), including Gulf red snapper (Patterson et al. 2001a, 2008; Cowan et al. 2003; Patterson 2007).

Otoliths, or earstones, have no living tissue in their structure. Therefore, unlike bone or scales, they are acellular and metabolically inert once formed (Campana and Thorrold 2001). Elements or stable isotopes from water or food that fish encounter are incorporated into the otolith matrix and retained as part of a permanent record of environmental exposure (Campana 1999). Analysis of the chemistry of an adult otolith's core material (i.e., the portion of an otolith formed prior to the first annulus) reveals chemical signatures imparted during the juvenile stage. These chemical signatures can be assayed from a thin section of an adult otolith by either extracting and analyzing the entire core region, or by microsampling a portion of the core via a narrow transect. A critical assumption of either of these general approaches is that the process of sectioning a whole adult otolith does not contaminate the chemical signature of its core, or if it does then a reliable method for removing such contamination can be developed. Secondly, in the case of mechanically extracting the entire core region, another assumption is that chemical signatures in the extracted core accurately reflect chemical signatures of the entire core region (i.e., the core section accurately reflects the entire signature that was present in a whole juvenile otolith). Microsampling core regions of adult otolith thin sections makes this second assumption more difficult to meet, whether subsampling occurs mechanically or via laser ablation, because narrow (and shallow with respect to laser ablation) transects across an otolith thin section effectively sample only a small percentage of the entire volume of the core, and concentrations of many constituents have been shown to be highly variable across core sections (Limburg et al. 2007; Elsdon et al. 2008).

The method we proposed to sample core regions of adult otoliths in the current study included preparing transverse sections of adult otoliths and then mechanically extracting their cores with a Micromill precision drilling instrument. However, we first tested whether extracting otolith cores in this manner significantly affected their chemical signatures. A series of experiments was conducted to test assumptions about extracting juvenile red snapper otolith chemical signatures from cores of adult otoliths, as well as to test if pulverizing otoliths, which is necessary for bulk analysis of otolith carbon and oxygen stable isotope signatures, introduced contamination into elemental signatures. The objectives of this component (now published in

Barnett and Patterson 2010) of the broader study were to test if element:Ca ratios or stable isotope delta values were significantly different between left and right whole otoliths of age-0 red snapper, to test if otolith element:Ca ratios or stable isotope delta values were significantly different between cored and whole age-0 otoliths, and to test if pulverizing introduced contamination or affected element:Ca ratios or stable isotope delta values. Herein, the term "core" refers to the portion of the otolith formed prior to the first annulus, which for red snapper basically represents the first six months of a fish's life (Patterson et al. 2001b; Wilson and Nieland 2001; Fischer et al. 2010). The primordium, or nucleus, is the central portion of the core and represents the material formed just prior to hatching and within the first several days of life.

Methods

Age-0 red snapper were sampled with otter trawls during October and November 2004 and 2005 from the northern Gulf during the National Marine Fisheries Service (NMFS) Fall Groundfish Survey. Red snapper were sorted from the overall catch, frozen in plastic bags onboard sampling vessels, and transferred to the University of West Florida Fisheries Laboratory for processing. Fish were thawed in the laboratory, measured to the nearest mm standard length (SL), and weighed to the nearest 0.01 g. Right and left sagittae were extracted from each fish with acid-leached glass probes and Teflon forceps. Each otolith was cleansed of organic tissue, rinsed with distilled water and placed under a Class 10 laminar flow cabinet (i.e., clean hood) for drying. Samples were allowed to air dry for at least 24 hours and then weighed to the nearest 0.1 mg.

A series of six experiments was conducted to test for potential effects of coring and pulverizing otoliths on otolith element:Ca ratios (Ba:Ca, Li:Ca, Mg:Ca, Mn:Ca, and Sr:Ca) and stable isotope delta values ($\delta^{13}C$ and $\delta^{18}O$). Two experiments were conducted to test for differences in elemental (experiment 1) or stable isotope (experiment 5) signatures between right and left otoliths to ensure side of fish head would not be a source of significant variance in later tests of the effects of coring and pulverizing (Table 1). Other experiments involved coring or coring and pulverizing otoliths to test the effects of those processes on chemical signatures. To sample otolith cores, the posterior and anterior ends of the distal side of left otoliths first were affixed to a microscope slide with Loctite Super Glue Control Gel. Core sections were removed by cutting transverse sections (Fig. 2A,B) through whole otoliths with an IsoMet low-speed saw. Width of sections (~1.5 mm) was standardized by using nylon spacers placed between two diamond saw blades. Due to the convex shape of sagittae, the locations of attachment to microscope slides, and the arrangement of saw blades, extracted core sections never came in contact with the glue used to affix otoliths to slides.

Otolith samples were cleaned prior to elemental or stable isotope analysis. Whole otoliths or otolith cores first were flooded with 1% ultrapure HNO_3 for 30 seconds to oxidize any material adhering to their surface. Clean otolith samples were flooded repeatedly with 18.3 MΩ cm^{-1} double deionized water ($DDIH_20$) to remove acid, and then placed under a Class 10 clean hood to air dry for at least 24 hours prior to reweighing. Following this second weighing, whole otoliths and otolith cores that were to be pulverized were transferred to acid-leached agate mortars in which samples were ground to a fine, homogenized powder with acid-leached agate pestles.

Otoliths processed for elemental analysis were dissolved in acid-leached high density polyethylene (HDPE) vials by adding a volume of 1% ultrapure HNO_3 to achieve a dilution factor of approximately 1,000x. Dissolution appeared to be complete within 1 hour, but samples were not manipulated for at least 24 hours once acid digestion began. Aliquots (5 ml) of otolith solutions were analyzed with a Finnigan MAT Element2 sector field-inductively coupled plasma-mass spectrometer (SF-ICP-MS) in the Department of Chemistry and Biochemistry at Old Dominion University. Otolith solutions were spiked with Indium at a concentration of 2.5 parts per billion (ppb) as an internal standard and then analyzed for ^{137}Ba, ^{48}Ca, ^{7}Li, ^{55}Mn, ^{25}Mg, and ^{86}Sr. Blanks were prepared from 1% ultrapure HNO_3 and processed through the same stages of sample preparation as sample solutions. Blanks were analyzed concurrently with sample solutions to estimate instrument limits of detection (LOD), which were estimated as three standard deviations of mean blank values. Instrument performance and matrix effects were checked by assaying elemental concentrations of an otolith standard reference material (SRM) prepared from adult red snapper otoliths (Sturgeon et al. 2005). Solutions of the SRM were prepared and analyzed similarly to red snapper otolith samples.

Pulverized otolith material that was processed for stable isotope analysis was transferred to microcentrifuge tubes and sealed. Subsamples (>1 mg) of homogenized pulverized otoliths were analyzed at the Stable Isotope Laboratory in the Department of Geology at The University of California at Davis with a Finnigan MAT 251 isotope ratio mass spectrometer (IR-MS). The instrument was calibrated against the International Atomic Energy Agency's carbonate standard, NBS-19. Accuracy of analytical runs was measured through routine analysis of an inhouse check standard which had been stringently calibrated against NBS-19. Results of IR-MS analysis are reported below in δ-notation $\{\delta X = [(R_{sample}/R_{standard})-1]*1{,}000$, where $X = {}^{13}C$ or ^{18}O and $R = {}^{13}C/{}^{12}C$ or $^{18}O/{}^{16}O\}$, and are expressed as per mil (‰) relative to the international carbonate standard, Vienna Peedee Belemnite.

Parametric assumptions of element:Ca and stable isotope data were tested prior to statistical analysis. Normality was tested with Ryan-Joiner tests (α=0.05) and homogeneity of variances was tested with F_{max} tests (α=0.05). Constituents that violated parametric assumptions were transformed either with ln or reciprocal transformations prior to statistical analysis. Differences in element:Ca ratios or stable isotope signatures between right and left otoliths then were tested with multivariate analysis of variance (MANOVA; Hotelling's paired T^2, α=0.05). When significant differences were found in overall element:Ca or stable isotope signatures between right and left otoliths in a given experiment, differences in individual constituents (i.e., element:Ca ratios or stable isotope delta values) were tested with paired t-tests (α=0.05). Also, if a significant treatment effect occurred, then treatment-specific residual values for each consituent were computed by subtracting the mean value of right otoliths from all right otolith samples and subtracting the mean value of left otoliths from all left otolith samples. Then, a Hotelling's paired T^2 test was computed on residual values. The purpose of these follow-up tests was to determine if significant differences between right and left otoliths, when found, were systematic within a given experiment, thus could be controlled by running statistical analyses on residual values versus raw data.

Results and Discussion

Sample sizes consisted of 10 otolith pairs for 5 of the 6 experiments (Table 1), with the remaining experiment having a sample size of nine. Fish had similar size (SL) distributions

among experiments, despite having been collected over a broad geographic range in the northern Gulf (Table 1). The acid cleaning process applied to otolith samples resulted in a mean (±standard error, SE) decrease in sample mass of 2.02% (±0.08) among all samples, with mean mass loss for cored samples being 1.99% (±0.16). Among cored samples, the mean percentage of whole otolith mass represented by core sections was 39.7%. Elemental concentrations were at least two orders of magnitude higher than the limit of detection for all elements analyzed in all samples. Standard reference material samples were within 5% of certified values for elements analyzed with SF-ICP-MS and stable isotope delta values were within 1% of accepted values for IR-MS analysis.

There was no significant difference in otolith element:Ca signatures (Hotelling's paired T^2, p=0.954; Fig. 3A) or stable isotope ratios (Hotelling's paired T^2, p=0.991; Fig. 3E) between whole right and whole left otoliths. Therefore, side of head did not affect chemical signatures and a treatment effect in subsequent experiments could be applied to an otolith from one side of fish's head, with the otolith from the other side serving as a control. A further implication of this finding is that chemical signatures of cores extracted later from sectioned adult otoliths would not be affected by the side of the head from which the otolith was extracted.

Pulverizing otoliths did not affect (i.e., contaminate) their element:Ca signatures, as there was no significant difference between whole right versus pulverized left otoliths in experiment 2 (Hotelling's paired T^2, p=0.726; Fig. 3B). This is important because when examining the core chemistry of sub-adult and adult fish, a thin section (~0.5 mm) of one otolith must be prepared for aging, while the second otolith is sectioned (~1.5 mm) and cored to examine core chemistry. Therefore, the core of this second otolith must be pulverized so a portion of the powder can be set aside to conduct IR-MS analysis, while the remaining powder is dissolved for solution-based SF-ICP-MS analysis. The results of experiment two indicate this added step of pulverization does not introduce contamination into elemental signatures if otolith cores are appropriately cleaned once extracted and clean instruments are used when pulverizing cores. Clearly, mechanically removing the core of an otolith is a destructive process which carries with it the real potential of introducing contamination to otolith chemical signatures. Our results indicate that the decontamination steps employed resulted in no issues of contamination from the saw blades used to section otoliths, as well as no issues of contamination from pulverizing.

Collectively, results from experiments three (Hotelling's paired T^2, p=0.015; Fig. 3C), four (Hotelling's paired T^2, p=0.167; Fig. 3D), and six (Hotelling's paired T^2 , p=0.007; Fig. 3F), indicated that coring had a significant effect on otolith chemical signatures. Subsequent univariate (paired t-test) analysis of differences in individual otolith chemical constituents between whole right and cored left otoliths from experiment three indicated that the significant multivariate difference was due to differences in Ba:Ca, Mn:Ca, and Li:Ca between whole right and cored left otoliths (paired t-tests, $p \leq 0.012$, respectively). While no significant difference existed in whole right versus cored and pulverized left otoliths in experiment four (Fig. 3D), the p-value from the paired Hotelling's T^2 test was nearly significant and there clearly was increased variability between right and left otolith values versus the nearly 1:1 relationship seen in results from experiment one (Fig. 3A). Lastly, the highly significant result from experiment six was due to a systematic difference of slightly higher $\delta^{13}C$ and $\delta^{18}O$ values in whole right versus cored left otoliths (paired t-test, p=0.007 and p=0.003, respectively).

Results from experiment three indicate that Ba:Ca and Mn:Ca ratios were consistently higher in cored left versus whole right otoliths, while Li:Ca had the opposite pattern. Brophy et al. (2004) reported otoliths of larval Atlantic herring, *Clupea harengus*, and sprat, *Sprattus*

sprattus, had higher Mn:Ca values near the primordium than in the rest of the otolith and concluded that potential maternal, physiological, or matrix effects all may contribute to confound the environmental signal of Mn:Ca in otolith cores. Similarly, Ruttenberg et al. (2005) reported Ba:Ca and Mg:Ca, as well as Mn:Ca, were elevated in the primordium of six species of larval fishes sampled from different regions of the Pacific Ocean. Therefore, systematic differences in Ba:Ca and Mn:Ca ratios we report between whole and cored juvenile red snapper otoliths most likely resulted from a disproportionate representation of the primordium in cored otoliths relative to the entire volume of whole otoliths (Limburg et al. 2007).

Differences in stable isotope signatures between whole right and cored left otoliths (experiment six) represented an even greater coring effect than that observed for element:Ca ratios. Systematically lower $\delta^{13}C$ and $\delta^{18}O$ values in cored versus whole otoliths may also reflect ontogenetic effects, as we infer above for differences observed in element:Ca ratios. Weidman and Millner (2000) reported that otolith primordia of Atlantic cod, *Gadus morhua*, contained lower $\delta^{13}C$ values which increased away from the primordium. Thus, they concluded that ontogenetic trophic shifts in feeding ecology were recorded in otolith $\delta^{13}C$. Thorrold et al. (1997) also reported that fractionation of $\delta^{13}C$ in reared Atlantic croaker, *Micropogonias undulatus*, was strongly influenced by metabolism, while $\delta^{18}O$ has been shown to be deposited in otoliths in near equilibrium with ambient $\delta^{18}O$ in water (Kalish 1991; Patterson et al. 1993; Thorrold et al. 1997). However, $\delta^{18}O$ in seawater is strongly correlated with water temperature (Jouzel et al. 1994). Therefore, the apparent ontogenetic effect observed in $\delta^{18}O$ values of age-0 red snapper otoliths likely reflected the range in temperatures fish encountered in the four to five months between the time they were spawned (May through July) until they were captured (October and November).

It was apparent before conducting this set of experiments that it would be imperative to be able to correct for any differences in chemical signatures we observed between whole and cored otoliths. The fact that differences in Ba:Ca, Li:Ca, Mn:Ca, $\delta^{13}C$, and $\delta^{18}O$ between whole and cored otoliths appeared to be systematic (Fig. 3C,F) provided some indication that a systematic correction would be possible such that the variance in signatures was captured even if shifts in mean values occurred prior to bias-correction. Our approach was to compute residuals of whole right otoliths by subtracting the mean value from observed values for each otolith constituent and then testing for differences between those residual values and ones likewise computed for cored left otoliths. This was done for element:Ca ratios measured in experiment three and stable isotope ratios measured in experiment six. The results were that no statistically significant differences existed between whole right and cored left otoliths in elemental signatures (Hotelling's paired T^2, $p=0.992$; Fig. 4A) or stable isotope signatures (Hotelling's paired T^2, $p=0.996$; Fig. 4B) when residuals were tested. Thus, computing models with residual values removed the systematic bias associated with coring. Thorrold et al. (2001) similarly employed residuals of region-specific age-0 weakfish, *Cynoscion regalis*, otolith element:Ca ratios and stable isotope values when parameterizing maximum likelihood models that were computed to estimate the source of recruits to offshore fisheries along the US Atlantic coast. However, their rationale for modeling residual values was not to control for potential ontogenetic effects, but was necessary due to analyzing element:Ca ratios of juveniles with solution-based ICP-MS versus laser-ablation ICP-MS (LA-ICP-MS) analysis of adult otolith core material.

Applying otolith chemical signatures of juveniles as natural tags to source adults to nurseries or to estimate population connectivity has become widespread in fisheries ecology. However, the pervasiveness of reports of ontogenetic effects on otolith chemical signatures begs

the question as to what is the best approach for assaying the core chemistry of adult otoliths. Results reported here indicate that raw element:Ca or stable isotope values of otolith cores were systematically biased relative to whole otoliths for age-0 red snapper, but also that ontogenetic effects could be controlled for by examining residuals of core signatures. Rooker et al. (2008), on the other hand, reported that coring bluefin tuna, *Thunnus thynnus*, otoliths did not affect their $\delta^{13}C$ or $\delta^{18}O$ values, but such assumption testing has been reported rarely in the literature.

Overall, results from this component of our study indicate chemical signatures in mechanically-extracted cores of adult otoliths can be employed as proxies for red snapper nursery signatures that were incorporated into otoliths when fish were juveniles. While significant differences were found between whole and cored age-0 otoliths for several element:Ca ratios (Ba:Ca, Mn:Ca, and Li:Ca) or stable isotope delta values ($\delta^{13}C$ and $\delta^{18}O$), differences were systematic and could be controlled for by analyzing residual instead of actual values (e.g., Thorrold et al. 2001). The fact that differences in actual values were systematic between whole and cored otoliths implies that ontogenetic shifts occurred which were not fully captured in cores versus whole otoliths. When an adult otolith is cored to analyze its juvenile chemical signature, it is not possible to maintain the entire volume of the otolith material that was accreted during the early life period of interest. For example, the 1.5 mm sections of the age-0 red snapper otolith cores in the present study represented only approximately 40% of the mass, hence volume, of the formerly whole juvenile otolith. The material lost in the coring process came from the anterior and posterior portions of an otolith, which represented otolith material accreted more recently (i.e., later in the juvenile period) than the core material extracted as transverse sections. Therefore, a disproportionate amount of the primordium and early core was sampled in transverse sections (i.e., cores) than was present in whole juvenile otoliths. Therefore, elements and stable isotopes more susceptible to ontogenetic shifts (see above), due to either changing hydrographic or biological parameters, were significantly different in cores versus whole otoliths. The fact that no significant difference was found when residuals were analyzed indicates that ontogenetic effects can be controlled for in statistical analyses computed to source adults to nursery regions based on their core chemistry.

Estimating Sources of Red Snapper Recruits

Introduction

Among the most critical information needed for effective fisheries management of reef fishes are estimates of post-settlement site fidelity and, conversely, interpopulational mixing. Estimates of these population parameters are important because they affect recruitment to different habitats and regions, as well as the resiliency of populations to fishing mortality or habitat degradation (Cowen et al. 2000; Thorrold et al. 2001). To examine exchange among populations, fisheries ecologists traditionally have employed artificial tagging studies to estimate fish movement patterns. Due to the shortcomings of this approach, such as tag loss, tagging induced mortality, or low tag reporting rates, rates of site fidelity or mixing often are not estimable from tagging data, or are highly uncertain when estimates are computed. Furthermore, it is exceedingly difficult to determine nursery origin of adults with tagging studies because young fish may suffer high capture and tagging related mortality, and the cost of tagging a sufficient sample to test movement and site fidelity hypotheses is often prohibitive (Patterson and Cowan 2003).

A more recent and promising approach to estimating nursery source(s) or movement patterns of adult fishes is to use elemental and stable isotope signatures in otoliths, or ear stones, as natural biogeochemical tags of fish from different water bodies, geographic areas, or stocks (Patterson et al. 2001a, 2008; Thorrold et al. 1998, 2001; Elsdon et al. 2008). Otoliths are aragonite and protein structures that serve in the accustico-lateralis system of fishes. They grow as fish grow, are metabolically inert once formed, and incorporate minor and trace metals from surrounding water into their matrices as they accrete (Bath et al. 2000; Campana 1999; Mugiya et al. 1991; Simkiss 1974). Therefore, otolith microchemical analysis reveals the environmental history of fish and can be used as a natural tag (Patterson et al. 2001a, 2008; Thorrold et al. 1998, 2001).

Several authors have demonstrated that natural tags derived from otolith chemistry are effective tools for discriminating among stocks of adult fishes (Campana and Thorrold 2001; Elsdon et al. 2008), while others have shown that otolith chemistry may be an effective permanent tag of nursery habitat that can be used to estimate the source(s) of recruits to adult populations (Gillanders and Kingsford 2000; Thorrold et al. 2001; Rooker et al. 2008). For example, Thorrold et al. (1998, 2001) utilized elemental and stable isotope signatures in juvenile weakfish, *Cynoscion regalis*, otoliths as natural tags of natal estuaries along the east coast of the United States, and then employed the tags to estimate the population structure of adults. Results indicated site fidelity of adult weakfish to their natal estuaries, as well as significant population structure that was indecipherable with genetics approaches. Rooker et al. (2008) were able to distinguish Gulf of Mexico versus Mediterranean Sea nursery origin of juvenile Atlantic bluefin tuna, *Thunnus thynnus*, with 87% accuracy based on C and O stable isotope values, and then estimated connectivity of bluefin tuna populations on either side of the north Atlantic based on those signatures.

Otolith chemistry also has been employed previously to examine population connectivity and recruitment patterns in northern Gulf red snapper. Patterson et al. (2008) reported that otolith elemental signatures distinguished three [north central Gulf, northwestern Gulf, and southwestern (US) Gulf] nursery regions with a mean accuracy of approximately 80% for four out of five cohorts examined (1996-2000). Elemental signatures at the core of adult otoliths

sampled from the 1996, 1997, 1999, and 2000 cohorts in 2001 then were analyzed to estimate population connectivity among regions. Results indicated little mixing between the eastern and western Gulf but significant post-settlement movement of individuals between the northwestern and southwestern Gulf regions (Cowan et al. 2003; Patterson 2007). These findings are consistent with tagging data discussed above that indicated adult red snapper tagged off Alabama and the Florida Panhandle moved significant distances to the east and southeast but only one fish was reported west of the Mississippi River outflow. Moreover, results from this initial otolith chemistry study indicated population structure that may explain differences in growth and reproductive biology observed among regions (Cowan et al. 2003; Fischer et al. 2004; Gold and Salliant 2007; Patterson 2007; Jackson et al. 2007). While red snapper genetics studies generally have failed to find significant differences in gene frequencies among Gulf regions, Pruett et al. (2005) and Saillant et al. (2010) inferred that patterns observed in genetics data are consistent with Gulf red snapper constituting a marine metapopulation, a conclusion also drawn by Patterson (2007) in a review of red snapper post-settlement movement studies.

We expanded on earlier otolith chemistry work in the current study by examining otolith chemical signatures in juvenile red snapper from throughout the Gulf, including Mexican waters. Specific objectives were 1) to estimate the source(s) of recruits to the west Florida shelf; 2) to estimate connectivity between populations off southeast Texas and northeast Mexico; and, 3) to further examine mixing dynamics between populations east and west of the Mississippi River. First, natural tags of red snapper nursery regions on the continental shelf of US and Mexican portions of the Gulf were developed via analysis of otolith elemental chemistry and otolith stable isotope values. Resulting year class- and region-specific otolith chemical signatures then were employed to estimate the source(s) of recruits to regions in the US Gulf and to waters off northeastern Mexico. Implications for the recovering red snapper population on the west Florida shelf, as well as for populations in the western US Gulf are discussed.

Methods

Age-0 red snapper from the 2005-2007 year classes were sampled from 6 regions in the Gulf of Mexico (Fig. 1). Regional boundaries followed the rationale and approach of Patterson et al. (2008) for the northern Gulf. Two Mexican regions also were established, one from south of the US/Mexico border to south of Vera Cruz, Mexico, and the second one encompassing the Campeche Bank. The boundary between the northwest Gulf (NWG) region and the southwest Gulf (SWG) region was latitude 94.5° W. The boundary between the NWG and north central Gulf (NCG) region was latitude 89.0° W, while the boundary between the NCG and east Gulf (EG) regions was Cape San Blas, Florida (89.3° W). Age-0 fish were sampled in fall (October and November) in the 4 US Gulf regions, while Mexican samples typically were collected between December and March. Also, age-0 lane snapper, *Lutjanus synagris*, were sampled from regions EG, NCG, NWG, and SWG in fall 2005 to determine if their otolith chemical signatures could be used as a surrogate for age-0 red snapper signatures.

Samples were collected in the NCG, NWG, and SWG regions using otter trawls aboard the United States' National Oceanographic and Atmospheric Administration's R/V Oregon II or R/V Gordon Gunter during the National Marine Fisheries Service's (NMFS) Fall Groundfish Survey in each year. Trawl stations were selected by NMFS biologists with stratified random sampling and our sample sites were selected randomly from those stations. Fish were sub-

sampled from a given station's trawl catch with systematic random sampling after first ordering individuals according to length. Immediately following selection, fish were placed in plastic bags and frozen.

Sampling in the EG, MEX1, and MEX2 regions was opportunistic and haphazard. Study personnel had standing requests with the Florida Fish and Wildlife Research Institute's (FWRI) Baitfish Survey, NMFS's Small Pelagic Survey, and Shrimp Observers coordinated by Dr. Elizabeth Scott-Denton and employed by the Gulf and South Atlantic Fisheries Foundation (GSAFF) to retain any juvenile red snapper captured along the west Florida shelf. Any fish collected by personnel associated with those surveys/groups who collected juvenile red snapper for this study placed the fish in plastic bags and froze them at sea. Samples were later transferred to the Fisheries Laboratory at the University of West Florida (UWF).

A broad call for collaboration was put out to Mexican scientists prior to the beginning of the study to elicit their help in obtaining fish from regions MEX1 and MEX2. Unfortunately, only one scientist responded to our request and he was not in a position to help. Dr. Cowan's group at Louisiana State University (LSU) did make contact with Ms. Gabriel Martínez from the Fisheries Oceanography Laboratory at the University of Merida in Merida, Mexico. Ms. Martinez facilitated the collection of juvenile red snapper that were caught as shrimp trawl bycatch on the Campeche Bank (MEX2), as well as along the Mexican shelf between Tampico and Vera Cruz (MEX1). Otoliths from Mexican samples were extracted by Ms. Martinez and shipped to LSU prior to being forwarded to UWF for sample processing.

Frozen age-0 fish (both red snapper and the limited sample of lane snapper) were thawed in the laboratory at UWF, weighed to the nearest 0.01 g, and measured to standard length (SL). Sagittae were extracted using glass probes and polyethylene tweezers; all materials that came in contact with extracted otoliths were acid-leached and triple-rinsed with $DDIH_2O$. Extracted otoliths were scrubbed with a synthetic bristle brush, rinsed with $DDIH_2O$, and placed in acid-leached polyethylene vials to air-dry.

Otolith samples were cleaned prior to elemental or stable isotope analysis. Whole otoliths or otolith cores first were flooded with 1% ultrapure HNO_3 for 30 seconds to oxidize any material adhering to their surface. Clean otolith samples were flooded repeatedly with $DDIH_20$ to remove acid, and then placed under a Class 10 clean hood to air dry for at least 24 hours prior to reweighing. Following this second weighing, whole otoliths and otolith cores that were to be pulverized were transferred to acid-leached agate or glass mortars in which samples were ground to a fine, homogenized powder with acid-leached pestles.

Right otoliths were dissolved in acid-leached high density polyethylene (HDPE) vials by adding a volume of 1% ultrapure HNO_3 to achieve a dilution factor of approximately 1,000x. Dissolution appeared to be complete within 1 hour, but samples were not manipulated for at least 24 hours once acid digestion began. Aliquots (5 ml) of otolith solutions were analyzed with a Finnigan MAT Element2 sector field-inductively coupled plasma-mass spectrometer (SF-ICP-MS) in the Department of Marine Sciences at the University of Southern Mississippi. Otolith solutions were spiked with Indium at a concentration of 2.5 parts per billion (ppb) as an internal standard and then analyzed for ^{137}Ba, ^{48}Ca, ^{7}Li, ^{55}Mn, ^{25}Mg, and ^{86}Sr. Blanks were prepared from 1% ultrapure HNO_3 and processed through the same stages of sample preparation as sample solutions. Blanks were analyzed concurrently with sample solutions to estimate instrument limits of detection (LOD), which were estimated as three standard deviations of mean blank values. Instrument performance and matrix effects were checked by assaying elemental concentrations of an otolith standard reference material (SRM) prepared from adult red snapper otoliths

(Sturgeon et al. 2005). Solutions of the SRM were prepared and analyzed similarly to red snapper otolith samples.

Left otoliths were pulverized with acid-leached glass or agate mortars and pestles and then transferred to microcentrifuge tubes and sealed. Subsamples (>1 mg) of homogenized pulverized otoliths were analyzed at the Stable Isotope Laboratory in the Department of Geology at The University of California at Davis with a Finnigan MAT 251 isotope ratio mass spectrometer (IR-MS). The instrument was calibrated against the International Atomic Energy Agency's carbonate standard, NBS-19. Accuracy of analytical runs was measured through routine analysis of an inhouse check standard which had been stringently calibrated against NBS-19. Results of IR-MS analysis are reported below in δ-notation $\{\delta X=[(R_{sample}/R_{standard})-1]*1{,}000$, where $X=^{13}C$ or ^{18}O and $R=\ ^{13}C/^{12}C$ or $^{18}O/^{16}O\}$, and are expressed as per mil (‰) relative to the international carbonate standard: Vienna Peedee Belemnite (V-PDB).

Correlation analysis was conducted between element:Ca ratios or stable isotope delta values and SL to test if significant linear relationships existed between otolith constituents and fish size. Any significant correlations were statistically removed from constituents by subtracting the slope of the least squares linear relationship. Parametric assumptions of age-0 element:Ca and stable isotope data then were tested prior to statistical analysis. Normality was tested with Ryan-Joiner tests ($\alpha=0.05$) and homogeneity of variances was tested with F_{max} tests ($\alpha=0.05$). Element:Ca ratios that violated parametric assumptions were transformed with ln transformations prior to statistical analysis. Regions NCG, NWG, and SWG were the only ones sampled each year of the study for age-0 fish, thus differences in chemical signatures only were tested among those regions, as well as among year classes, with MANOVA ($\alpha=0.05$). Differences in individual constituents for those same factors were tested with analysis of variance (ANOVA, $\alpha=0.05$). The ability to distinguish nursery regions with chemical signatures was evaluated with year class-specific and combined year class discriminant function analysis. First, stepwise linear discriminant function (LDF) models were computed with Proc STEPDISC in SAS (SAS Institute, Inc., 2004). Then, classification accuracy of individual LDF models was estimated with the jackknife crossvalidation procedure in SAS's PROC DISCRIM.

Sub-adult and adult red snapper were sampled in summer 2006-2008 onboard NMFS scientific surveys, by NMFS port agents, and by personnel of the UWF and LSU Fisheries Laboratories throughout the northern Gulf, and by Mexican scientists in Mexican waters. Sampled fish were measured to fork and total length (FL, TL) and then both sagittae were extracted, rinsed free of associated tissue, and stored dry in paper coin envelops or plastic vials. The left sagitta of each sample was aged at either UWF or LSU following the methods of Patterson et al. (2001b) and Wilson and Nieland (2001). Following aging, fish were subsampled with stratified random sampling to select up to 50 fish per region per year class in each summer of sampling for coring and chemical analysis. For example, only fish from the 2005 year class were available to be sampled in summer 2006, but both the 2005 and 2006 year classes were sampled in summer 2007 when fish from those cohorts were 2 year olds and 1 year olds, respectively. All three year classes were available for sampling in summer 2008.

Right otoliths selected for chemical analysis were embedded in epoxy, affixed to microscope slides and then a transverse section was extracted, capturing the core, with an Isomet saw fitted with twin diamond blades separated by a 1.5 mm nylon spacer. Anterior end and posterior ends of otolith sections with associated epoxy were affixed to an acid-leached nylon washer with Loctite Super Glue Control Gel, such that neither the glue nor the washer came in contact with the core region of the otolith. The washer itself was affixed to an acid-leached

microscope slide. Serial passes then were made with a Micromill precision drilling instrument to cut a predetermined pattern out of otolith sections such that the age-0 core of sections was extracted (Fig. 2C,E,F). The pattern was determined by sectioning 20 age-0 otoliths collected in fall 2004 with the technique employed in the coring experiments described above. Age-0 otoliths were affixed to slides, core sections extracted, and then the 6 dimensions of the pattern shown in Fig. 2C were measured in core sections. The mean of each dimension among the 20 age-0 cores then was computed to derive the pattern shape.

Extracted cores were rinsed with $DDIH_2O$ to remove otolith dust and then stored in plastic vials. Prior to chemical analysis, any potential contamination was removed as described above for age-0 otoliths. Once dried and reweighed, cores were pulverized in acid-leached mortar and pestles and the resulting homogenized powder was split. Half the otolith powder from a given core was placed in an acid-leached HDPE vial and a volume of 1% ultrapure HNO_3 was added to achieve a dilution factor of approximately 1,000x. The other half of the powder was placed in a microcentrifuge tube for storage. Then, dissolved otolith core samples were analyzed with SF-ICP-MS, and powdered samples were analyzed with IR-MS, as described above for age-0 samples. Resulting element:Ca and stable isotope data were transformed exactly how constituents were transformed for age-0 samples prior to conducting maximum likelihood analysis to estimate the year class-specific source(s) of recruits to given region in a given sampling year. The HISEA maximum likelihood model described by Millar (1990) was used to estimate source(s) of recruits. Following the rationale provided above and discussed further in Barnett and Patterson (2010), the HISEA model was parameterized with year class-specific residual values of transformed and slope-corrected age-0 (when appropriate) data for each element:Ca ratio and stable isotope delta value. Therefore, three unique standard (rule) files were created in HISEA to estimate source(s) of recruits in samples of 2005, 2006, and 2007 year classes, respectively, later sampled as 1, 2, or 3 year-old fish. Constituent-specific residuals also were computed for sub-adult and adult core data for each year class in each year that it was sampled, such that 6 HISEA mix files were created, one each for the 2005 year class sampled in 2005, 2006, and 2007, one each for the 2006 year class sampled in 2007 and 2008, and one for the 2007 year class sampled in 2008. Models then were computed to estimate the source(s) of recruits to each region for each year class in each year of sampling.

Results and Discussion

A total of 2,117 juvenile red snapper was sampled across 6 Gulf regions during fall 2005-2007, and of those samples 432 were analyzed for otolith chemistry (Table 2; Fig. 5). No age-0 red snapper were captured in the FWRI Baitfish Survey nor in the NMFS Small Pelagics Survey, which greatly diminished our potential sampling effort on the west Florida shelf. The only age-0 fish that were sampled in the EG region came from GSAFF and NMFS observers. In 2005, the only EG samples came from observers working on pink shrimp boats operating west of Tampa. No EG fish were sampled in 2006, and the 2007 samples came from just north of the Dry Tortugas (Fig. 5). Attempts were made to work with personnel at the Galveston NMFS Laboratory, who control the spatial and temporal coverage of shrimp observer effort, to get greater coverage along west Florida in fall, but we were told that was not possible. We also pursued several leads among shrimp industry liaisons but had no success in procuring additional samples for broader EG coverage in 2005 and 2007, or any samples in 2006. It should be noted,

however, that the FGS trawl survey was expanded to cover shelf areas off the Florida Panhandle and along west Florida during fall 2008 and 2009. While we did not have the budget to run additional age-0 otolith chemistry samples from those year classes in this study, samples from all 4 northern Gulf regions collected in 2008 and 2009 have been archived.

Samples were relatively easy to obtain for the NCG, NWG, and SWG regions due to the annual FGS occurring in those regions. Mexican regions, however, proved to be as challenging as the EG region for sampling age-0 red snapper. No age-0 fish were sampled from regions MEX1 or MEX2 in 2005, but samples were available in 2006 (n = 103) and 2007 (n = 96) thanks to the efforts of Ms. Gabriel Martínez. Samples collected in region MEX2 came a few months later in the year in both 2006 and 2007 than did samples from the other regions. When examining region-specific size distributions, it also became apparent that fish from MEX2 were substantially larger than age-0 fish collected in the other regions (Fig. 6). No attempt was made to age these fish with daily growth increments, but being approximately 90 days older than fish captured in other regions alone would explain the 80-90 mm difference in mean size observed between MEX2 samples and those from the other regions. It also was clear that MEX2 fish had extreme values for some element:Ca ratios or stable isotope values (Fig. 7). When correlation analyses were performed between constituent values and fish SL, it was apparent that the large MEX2 fish were greatly influencing the results. Therefore, given the size and likely age disparity between MEX2 fish and fish from the other 5 regions, MEX2 were not included in subsequent analyses.

There was a significant difference in otolith chemical signatures among the NCG, NWG, and SWG regions (Table 3). While region and year class factors were not both significant for every constituent analyzed, they and their interaction were significant for most. Plots of mean region-specific mean constituent values reveal interregional patterns in otolith chemical signatures, but the data are not typified by the consistent interregional patterns as was reported by Patterson et al. (2008) for the NCG, NWG, and SWG Gulf regions sampled in 1996-2000. Part of the reason for that lack of continuity among year classes in the current study is that twice as many regions were sampled as previously, but also the EG and MEX1 regions were not sampled in each year of the study. One consistent pattern observed is that fish from the EG region tended to have low or high element:Ca ratios or stable isotope delta values relative to the other regions. This was also true of MEX1 fish, which may stem from the fact that both the EG and MEX1 regions had lower latitudes than fish from the other regions, as well as the fact that the regions in the northern Gulf have greater freshwater input given the Mississippi River and Mobile Bay drainages (Patterson et al. 2008). In fact, the most consistent trend in the age-0 data is the similarity in values between NCG and NWG regions among all constituents in each year of the study (Fig. 7).

Age-0 lane snapper (n = 120) collected in EG, NCG, NWG, and SWG regions in fall 2005 had TL distributions similar to age-0 red snapper collected in those regions (Fig. 8). However, there were substantial differences in otolith chemical signatures between species (Fig. 9). The largest differences were in Mg:Ca, Sr:Ca, and $\delta^{18}O$. Observed differences likely stem from the fact that lane snapper may first recruit to estuaries before moving offshore, while red snapper settle out of the plankton and then spend their entire lives over the shelf (Patterson et al. 2005; Patterson 2007; Wells et al. 2008). Sr:Ca is strongly influenced by salinity and to a lesser extent temperature, while Mg:Ca can be affected by both salinity and physiology, which is indirectly affected by water temperature (reviewed in Campana and Thorrold 2001 and Elsdon et al. 2008). Oxygen isotopes are incorporated into otolith aragonite in near equilibrium with water

$\delta^{18}O$ values, and water $\delta^{18}O$ is strongly controlled by temperature. Therefore, lower salinities and higher summer and fall temperatures typical of northern Gulf nearshore environments and estuaries likely drove differences observed between lane and red snapper otolith chemistry. Those differences preclude lane snapper otolith chemical signatures from serving as effective proxies for red snapper signatures. Perhaps vermilion snapper, *Rhomboplites aurorubens*, otolith chemical signatures might be better candidates to serve as proxies for red snapper, but attempts to collect age-0 vermilion snapper otoliths from a broad geographic range were unsuccessful in fall 2005 when samples were collected for these comparisons.

The stepwise discriminant function model building algorithm retained all otolith chemical constituents for the 2005 year class model, retained all constituents except Li:Ca in the 2006 model, and retained all constituents except Mg:Ca in the 2007 model. Mean classification accuracies of resulting LDF models were 87.3% in 2005, 75.3% in 2006, and 67.2% in 2007 (Fig. 10A-C). While the 2007 model's classification accuracy is nearly 20% lower than in 2005, the 2007 model was the only one that contained samples from all 4 US Gulf regions as well as MEX1, and the null for random assignment for a 5-region model is 20% accuracy per region. Perhaps a greater issue than the mean accuracy of the 2007 LDF model is the relatively poor classification of fish from the NCG and NWG regions in all three year class-specific models, with mean classification accuracy for these two regions among models being 67% and most of the misclassification error from the NWG being to the NCG and vice versa.

The model in which data from all year classes were modeled jointly produced a mean classification accuracy of only 49% among regions (Fig. 10D). Perhaps that should have been expected given the significant differences in age-0 chemical signatures among years, as well as the significant region*year interaction, but not having samples from all regions in all years necessitated at least attempting to model all year classes jointly in the case that a multi-year class model could be used to estimate nursery origin for years in which not all regions were sampled. Our conclusion from this exercise is that poor classification accuracy of the joint year class model would severely compromise the efficacy of such an approach.

A total of 2,905 sub-adult and adult red snapper was sampled from study regions during summer 2006-2008, and of those samples 1,123 otolith samples were cored and analyzed for otolith chemistry (Table 4). There are common trends among mean element:Ca ratios and stable isotope delta values among sampling regions and cohorts and across sampling years for the sub-adult and adult otolith core data (Fig. 11). For example, several element:Ca ratios, such as Ba:Ca, Mn:Ca, and Sr:Ca tend to have an inverted u-shaped pattern with lowest ratios for the EG and MEX1 regions and highest values for the SWG region. The opposite pattern exists for $\delta^{18}O$ and Li:Ca data, for which mean values tend to be high for EG and MEX1 regions and lowest for SWG. For constituents displaying either pattern type, means for the 3 central regions (NCG, NWG, and SWG) tend to cluster together.

The ultimate goal of this study was to estimate source(s) of red snapper recruits to Gulf regions based on otolith chemical signatures and plots of maximum likelihood estimates (MLE) of nursery origin reveal those results (Fig. 12). Beginning with plots of MLE results for age-1 fish of the 2005, 2006, and 2007 year classes sampled in 2006, 2007, and 2008, respectively, two distinct patterns emerge. First, MLEs indicate that age-1 EG fish were largely derived from that region, with the NCG secondarily indicated as a source of recruits to the EG. For age-1 fish in 2007, only approximately 50% of EG fish were estimated to have an EG nursery origin, with the remaining fish estimated to have been derived from the NCG and NWG in nearly equal proportions. However, it is important to recall that NCG and NWG fish were poorly

distinguished in LDF models due to similarities in age-0 otolith chemistry, a pattern that was consistent across year classes but particularly acute in 2006 age-0 fish. Therefore, it is uncertain what percentage of EG age-1 fish actually were derived from the NWG versus the NCG, if any.

Previous red snapper otolith chemistry results indicated limited movement in the first year of life (Cowan et al. 2003; Patterson 2007; Patterson et al. 2008), but results of the current study indicate more substantial movement among regions for the youngest fish. While MLEs of age-1 EG fish nursery origin indicate fish in that region were largely locally-derived, the second significant trend among regions for age-1 fish is the estimated importance of the NWG as a source of recruits to other regions. The NWG was estimated to be the most significant source of recruits to the SWG for the 2005 cohort sampled as age-1 fish in summer 2006, and that pattern was consistent for the 2005 year class as 2 and 3 year-old fish. For the 2006 cohort sampled in 2007, the NWG was estimated to be the most significant source of recruits to the NCG, NWG, and SWG. Again, a high degree of uncertainty exists in the connectivity between the NCG and NWG due to the low degree of distinctness in the their age-0 otolith chemical signatures. Nonetheless, the NWG appears to have been an important source of recruits to SWG, NWG, and NCG across the years and among year classes examined. Saillant and Gold (2006) and Saillant et al. (2010) estimated that the effective genetic population size of fish in this region is ten-fold greater than other US Gulf regions, and Mitchell et al. (2004) reported that catch rates of large adult red snapper in experimental longline sets along the outer shelf were nearly 15 times greater in the NWG than in the western portion of the NCG Gulf region. Therefore, uncertainty aside, patterns observed in MLEs of nursery origin that indicate the importance of the NWG as a source region of recruits are consistent with other sources of information, as well as with 2009 red snapper stock assessment results that indicated a truncated age distribution in the eastern versus western Gulf and a spawning potential ratio in the east that was just a fraction of that estimated for the west.

Patterns observed for the 2006 year class in both 2007 and 2008 only partially fit patterns discussed above. First, it should be noted that although age-1 and age-2 fish were sampled in the EG in summer 2007 and 2008, respectively, no nursery estimates were plotted for that region because MLEs of nursery origin necessarily indicate EG samples were derived from other regions because no age-0 EG samples were available for the 2006 year class. For the other US regions, age-1 fish sampled in 2007 were estimated to be largely locally derived, although about a third of NCG age-1 fish were estimated to have been produced by the NWG. Very few age-1 fish sampled in either the NWG or SWG were estimated to have been derived from MEX1 for this year class, a pattern that was also seen for the 2007 cohort. That is significant because otolith chemical signatures of age-0 MEX1 samples were distinct and had among the highest LDF classification accuracies in both 2006 and 2007, yet MEX1 is not estimated to have been a significant source of recruits to western US Gulf regions. Higher percentages of MEX1 fish were estimated to have contributed recruits to US regions for age-2 fish sampled in 2008, but the near uniform pattern of equal source region estimates across sampled regions is odd and unexplainable at this point. Perhaps the lack of EG age-0 samples for the 2006 cohort forced model estimates that were unstable or inaccurate for age-2 fish. We were hopeful that the LDF model derived from all age-0 samples across year classes would enable us to effectively borrow the EG age-0 signal from the 2005 and 2007 year classes to estimate source regions for the 2006 year class, but the low classification accuracy of that model precluded such an approach.

Maximum likelihood estimates of region-specific red snapper origin have important implications for red snapper population structure and management. First, there is little evidence

of connectivity between Mexican and US waters, which probably should not be surprising given estimates that the Mexican stock is severely overfished and the fact that it is centered on the Campeche Bank in the southern Gulf (Garcia et al. 2002). Second, it appears that recruitment of small, young fish to the EG region is largely due to self-recruitment within that region, although other regions were estimated to supply recruits even for age-1 fish. Tagging studies have routinely shown some fish tagged off Alabama and the western Florida Panhandle move eastward and are recaptured by fishermen on the west Florida Shelf south of Cape San Blas, Florida. However, Patterson et al. (2001c) reported that small, young red snapper were less likely to display movement and that fish size affected distance moved as well, a pattern that also was observed by Addis et al. (2008). Otolith chemical signatures reported here for the 2005 and 2007 year classes should be employed in subsequent years to examine cohort-specific patterns of recruitment to the west Florida shelf as the red snapper population there continues to respond to management measures designed to rebuild the stock. Furthermore, more complete spatial coverage of sampling for the 2008 and 2009 year classes along the west Florida shelf should provide even more robust otolith chemical signatures to examine recruitment dynamics and interpopulational mixing for those cohorts.

Results from a variety of previous studies provided the impetus to begin to assess red snapper populations in the eastern versus western Gulf separately in 2005, with the Mississippi River serving as the dividing line stock components. Data from genetics studies indicated some population structure existed, and that the red snapper population in the NWG had a ten-fold greater genetic effective population size than other regions (Pruett et al. 2005; Saillant and Gold 2006; Saillant et al. 2010). Results from age and growth and reproductive biology studies also indicated regional differences in population demographics (Fischer et al. 2004; Jackson et al. 2007). And lastly, post-settlement movement estimates from conventional tagging data and previous otolith chemistry work indicated substantial interpopulational mixing either east or west of the mouth of the Mississippi River, but little exchange between east and west (Patterson et al. 2001c, 2008; Cowan et al. 2003; Patterson 2007; Strelcheck et al. 2007; Addis et al. 2008). Therefore, caution should be applied when interpreting results of this study that suggest interpopulational mixing of small, young red snapper occurred across the mouth of the Mississippi River, especially given the low accuracy described above in distinguishing age-0 fish from the NCG and the NWG with otolith chemical signatures.

Red snapper movement has been shown to be affected by hurricanes and the active hurricane season in 2005 (Katrina, Rita) may have affected recruitment patterns observed here (Watterson et al. 1998; Patterson et al. 2001c). However, little exchange of fish between the eastern and western Gulf was apparent for the 2005 year class when fish were sampled as 1, 2, or 3 year-olds (Fig. 12). It may be that as the US Gulf red snapper stock as a whole increases in size it is expanding outward from its center of abundance off Louisiana and estimates produced here are beginning to capture that signal. Most of the recent increase in Gulf red snapper spawning potential ratio (SPR) is estimated to have occurred in the western versus eastern Gulf (SEDAR 2009), and that pattern likely will remain into at least the near future for a couple reasons. First, the truncated age structure estimated for the eastern Gulf means it is unlikely that egg production will increase as rapidly as it has been estimated to increase in the western Gulf. Secondly, the current management paradigm for Gulf red snapper is to assess eastern and western stock components separately but then base estimates of stock status on the overall stock. The implications of this approach are that the western component is projected to increase in size well above 26%SPR, which is the biomass at maximum sustainable marginal yield threshold adopted

by the Gulf of Mexico Fishery Management Council (Gulf Council), while the eastern component of the stock would never achieve higher than a 18%SPR, even at full stock recovery. Unless the Gulf Council adopts measures to assess *and* manage eastern and western components of the Gulf Stock separately, the western component will likely continue to be much larger than the eastern component into the future. Whatever directions management and stock recovery rate take, results presented here indicate that otolith chemistry can provide an effective tool to monitor stock recovery and its effect on recruitment dynamics among US Gulf regions.

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Tables and Figures

Table 1. Coring experiments designed to test effects of coring and pulverizing on juvenile red snapper otolith elemental or stable isotope signatures.

Experiment	Test	Analysis	Objective	n	Sampling Location	Mean SL (mm) ± SE
1	Whole right versus whole left otolith	SF-ICP-MS	To test if there were significant differences in elemental signatures between left and right otoliths	10	N28° 32.99' W90° 43.73'	102.9 ± 2.3
2	Whole right versus pulverized left otolith	SF-ICP-MS	To test if pulverizing otoliths contaminated elemental signatures	10	N27° 32.20' W96° 40.72'	103.7 ± 2.4
3	Whole right versus cored left otolith	SF-ICP-MS	To test if coring significantly affected otolith elemental signatures	9	N29° 43.78' W88° 15.37'	99.5 ± 4.8
4	Whole right versus cored and pulverized left otolith	SF-ICP-MS	To test if coring and pulverizing significantly affected otolith elemental signatures	10	N28° 32.85' W93° 58.86'	105.8 ± 4.5
5	Whole right versus whole left otolith	IR-MS	To test if there were significant differences in stable isotope signatures between left and right otoliths	10	N27° 07.59' W96° 49.01'	99.7 ± 1.5
6	Whole right versus cored and pulverized left otolith	IR-MS	To test if coring and pulverizing significantly affected otolith stable isotope signatures	10	N28° 32.85' W93° 58.86'	96.5 ± 3.8

Table 2. Region specific sample sizes of age-0 red snapper captured among 6 Gulf of Mexico regions from 2005 through 2007. GSAFF = Gulf and South Atlantic Fisheries Foundation; NMFS = National Marine Fisheries Service; and, UWF = University of West Florida.

Year	Region	Sampling Mode	Fish Sampled	Sample Sites	Size Range mm TL	Samples Analyzed for Otolith Chemistry	Sites Analyzed for Otolith Chemistry
2005	EG	GSAFF Observer	20	16	76 - 106	20	9
	NCG	Scientific Survey	130	19	55 - 150	30	11
	NWG	Scientific Survey	90	18	83 - 150	30	11
	SWG	Scientific Survey	186	23	61 - 150	30	10
	MEX1	--	--	--	--	--	--
	MEX2	--	--	--	--	--	--
2006	EG	--	--	--	--	--	--
	NCG	Scientific Survey, UWF Trawl Sampling	118	19	54 - 150	30	9
	NWG	Scientific Survey	159	56	54 - 150	30	13
	SWG	Scientific Survey	208	39	71 - 150	30	10
	MEX1	Mexican Scientists	68	1	95 - 190	30	1
	MEX2	Mexican Scientists	35	2	130 - 230	30	2
2007	EG	NMFS Observer	67	6	64 - 150	30	2
	NCG	Scientific Survey	142	11	65 - 146	30	7
	NWG	Scientific Survey	164	47	62 - 146	30	14
	SWG	Scientific Survey	644	68	53 - 150	30	15
	MEX1	Mexican Scientists	25	2	75 - 220	22	2
	MEX2	Mexican Scientists	61	1	60 - 230	30	1

Table 3. Results of MANOVA and ANOVA models computed to test for differences in otolith chemical signatures among regions and sampling years for age-0 red snapper sampled in the Gulf of Mexico in 2005 to 2007. The statistic computed in MANOVA models was Pillai's Trace, and mean square error (from Type III sum of squares) in ANOVA models.

Model	Statistic Value	F-Value	Degrees of Freedom	Prob > F
MANOVA				
Region	0.95	29.06	14; 452	<0.001*
Year	0.30	5.79	14; 452	<0.001*
Region*Year	0.38	4.64	21; 681	<0.001*
Ba:Ca ANOVA				
Region	6.86	56.45	2; 231	<0.001*
Year	0.29	2.42	2; 231	0.091
Region*Year	0.45	3.67	3; 231	0.013*
Li:Ca ANOVA				
Region	0.30	12.76	2; 231	<0.001*
Year	0.23	9.89	2; 231	<0.001*
Region*Year	0.13	5.38	3; 231	0.001
Mg:Ca ANOVA				
Region	0.51	21.53	2; 231	<0.001*
Year	0.02	0.95	2; 231	0.389
Region*Year	0.13	5.30	3; 231	0.002*
Mn:Ca ANOVA				
Region	54.2	288.6	2; 231	<0.001*
Year	1.69	9.02	2; 231	<0.001*
Region*Year	0.54	2.87	3; 231	0.037*
Sr:Ca ANOVA				
Region	0.07	18.14	2; 231	<0.001*
Year	0.02	4.30	2; 231	0.015*
Region*Year	0.01	1.55	3; 231	0.202

Table 3. continued.

Model	Statistic Value	F-Value	Degrees of Freedom	Prob > F
δ^{13}C ANOVA				
Region	9.14	50.80	2; 231	<0.001*
Year	0.29	1.61	2; 231	0.202
Region*Year	0.83	4.63	3; 231	0.004*
δ^{18}O ANOVA				
Region	8.22	128.9	2; 231	<0.001*
Year	0.68	10.64	2; 231	<0.001*
Region*Year	0.27	4.23	3; 231	0.006*

Table 4. Region specific sample sizes of sub-adult and adult red snapper captured among six Gulf of Mexico regions in summer 2006 through 2008. UWF = University of West Florida.

Year	Region	Fish Sampled	Sampling Mode	Sites Sampled	Size Range mm TL	Samples Aged	Samples Cored and Analyzed
2006	EG	6	Scientific Survey	3	266 - 518	6	3
	NCG	105	Scientific Survey, other UWF research	19	152 - 292	104	63
	NWG	220	Scientific Survey	54	151 - 325	167	52
	SWG	160	Scientific Survey	39	151 - 293	142	52
	MEX1	35	Mexican Landings	1	250 - 280	31	18
	MEX2	28	Mexican Landings	1	230 - 260	27	3
2007	EG	49	Scientific Survey, Rec (by me)	9	201 - 460	49	39
	NCG	156	Scientific Survey, Comm Landings, other UWF research	25	177 - 410	116	85
	NWG	294	Scientific Survey, Rec Landings	82	151 - 443	147	111
	SWG	290	Scientific Survey, Rec Landings	35	152 - 366	128	104
	MEX1	119	Mexican Landings	2	240 - 380	110	81
	MEX2	142	Mexican Landings	1	240 - 480	132	4
2008	EG	66	Scientific Survey, Rec&Comm Landings, Observers	13	152 - 528	66	58
	NCG	387	Scientific Survey, Rec&Comm Landings, other UWF research	29	152 - 528	182	150
	NWG	456	Scientific Survey, Rec&Comm Landings	42	151 - 699	248	150
	SWG	392	Scientific Survey, Rec&Comm Landings	49	151 - 682	206	150
	MEX1	--	--	--	--	--	--
	MEX2	--	--	--	--	--	--

Figure 1. Map of six sampling regions in the northern Gulf of Mexico (Gulf) where age-0 red snapper were sampled in 2005, 2006, and 2007, and sub-adult and adult fish were sampled in summer 2006, 2007, and 2008. Region abbreviations are EG = eastern Gulf, NCG = north central Gulf, NWG = northwestern Gulf, SWG = southwestern (US) Gulf, MEX1 = Mexico 1, and MEX2 = Mexico 2.

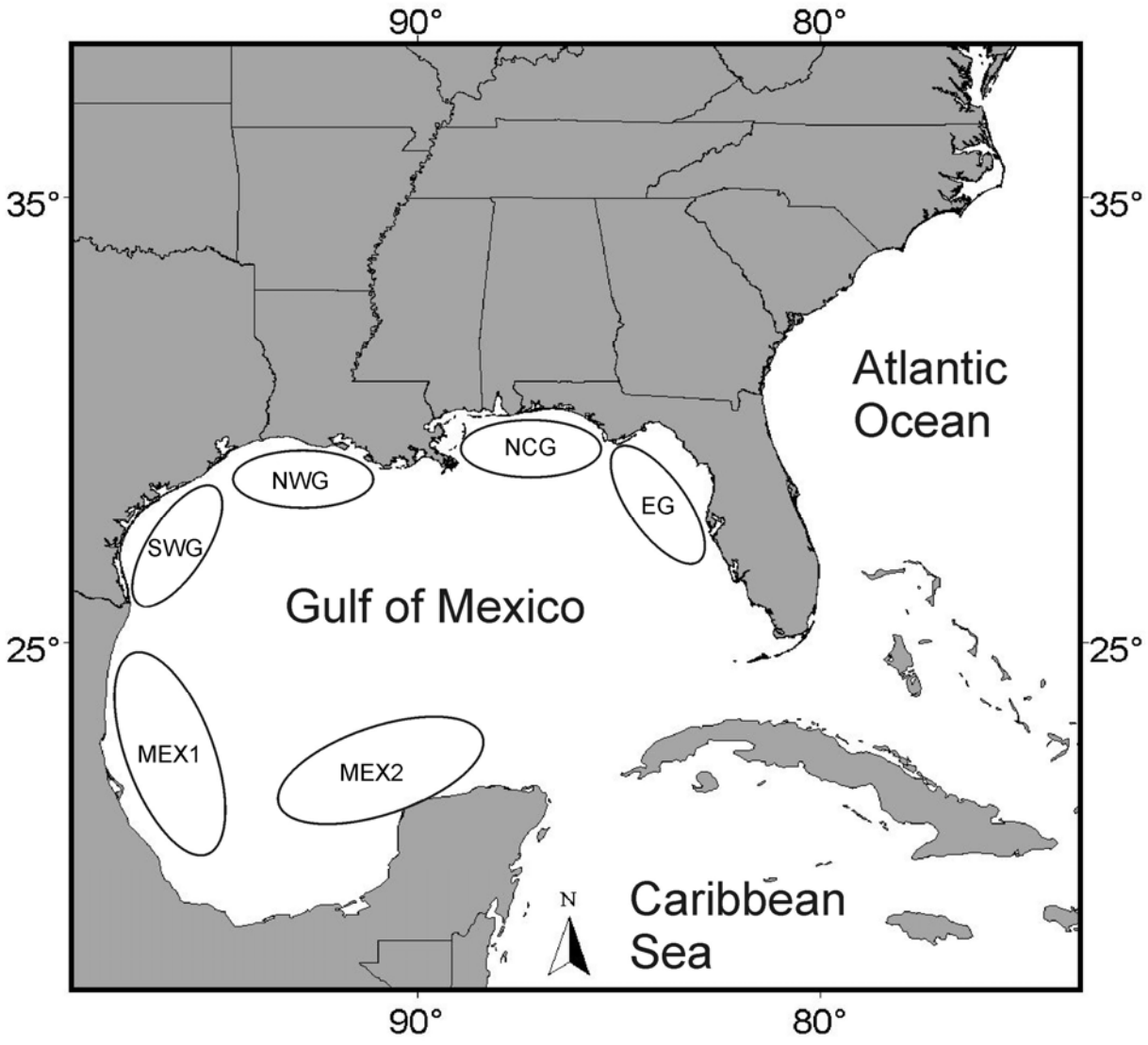

Figure 2. Digital images of whole red snapper sagittae, otolith sections, and extracted cores. All images are shown at a common scale, with bars associated with each image scaling to 1 mm in length. Image A is an age-0 otolith, with dashed vertical lines indicating a 1.5 mm wide transverse core section centered on the otolith's primordium. Image B is the 1.5 mm core extracted from A after affixing the anterior and posterior portions of A, proximal side up (as shown), to a microscope slide and cutting a core section with a pair of diamond saw blades separated by a 1.5 mm nylon spacer. A series of similar sections of age-0 otoliths (n = 20) was used to produce a template (C) to extract cores from sub-adult and adult red snapper. Image D is a right sagitta from a 563 mm total length red snapper, with dashed vertical lines indicating a 1.5 mm wide transverse core section centered on the otolith's primordium. Image E is the transverse section extracted from otolith D, viewed under reflected light, with annual opaque zones indicated with black dots and a white outline of the template used to extract the age-0 core with a Micromill. Image F is the resulting extracted core.

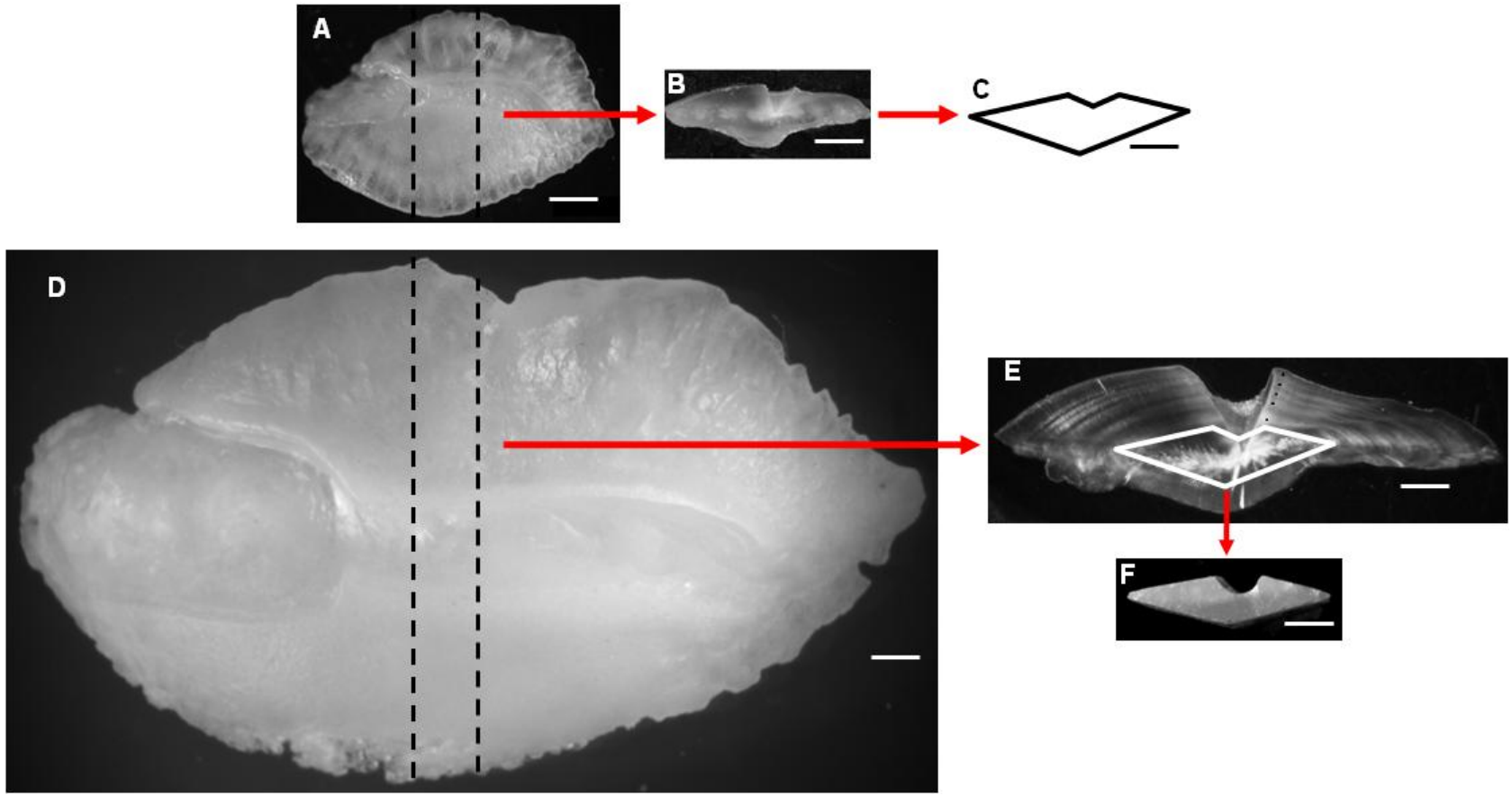

Figure 3. Results of experiments designed to compare element:Ca ratios or stable isotope delta values between right and left sagittal otoliths and to test the effects of coring and pulverizing on age-0 red snapper chemical signatures. Panels A-F present results from experiments one through six as presented in Table 1. Legend for Panels A-D is on Panel A; units are μmolmol^{-1} for Ba:Ca, Li:Ca and Mn:Ca, 100 μmol mol^{-1} for Mg:Ca, and mmol mol^{-1} for Sr:Ca. Legend for Panels E and F is on Panel E; units are ‰. Dashed lines indicate the line of 1:1 agreement.

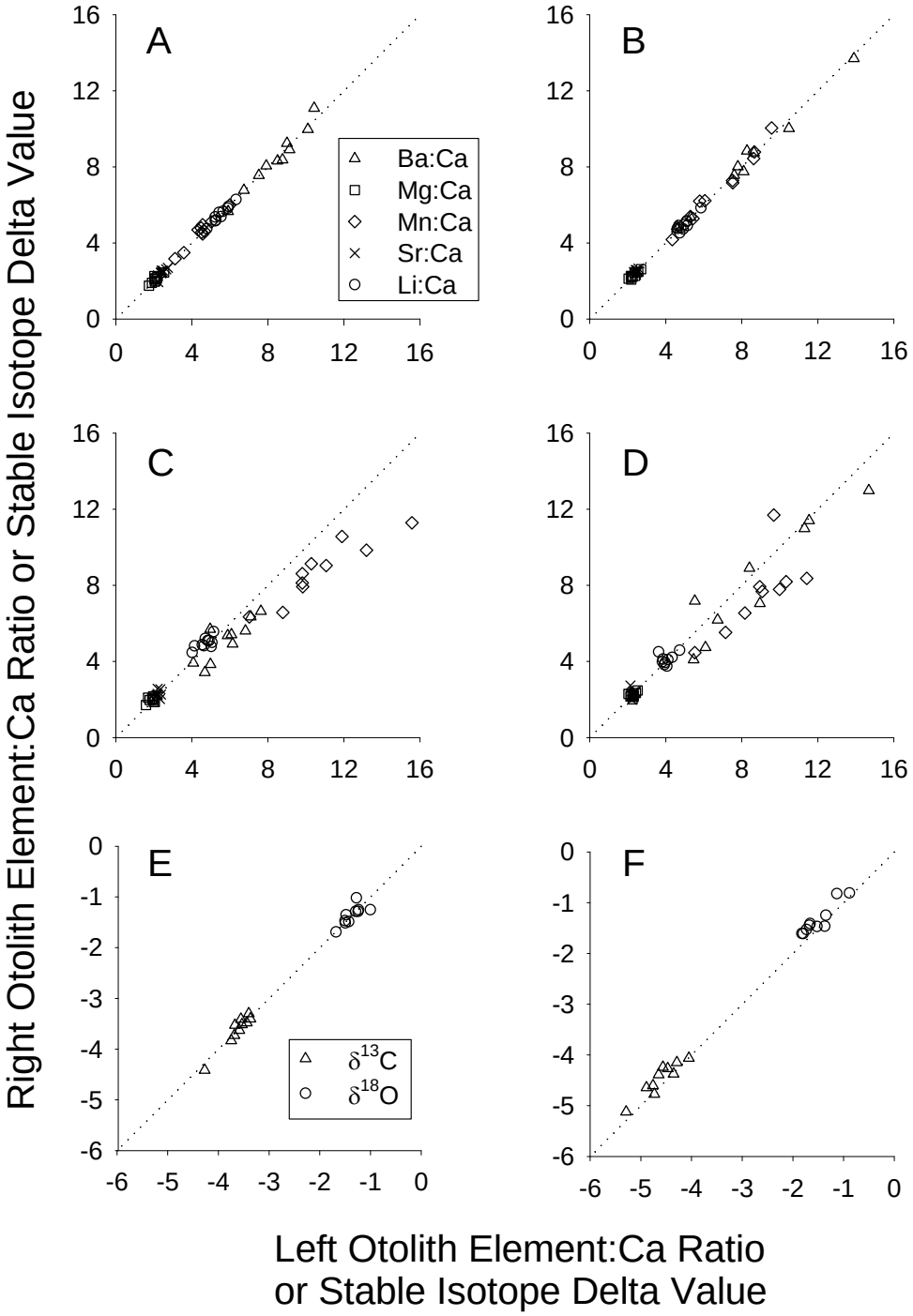

Figure 4. Plots of A) residual values of right versus left otolith element:Ca ratios from experiment three and B) residual values of pulverized right versus cored and pulverized left otolith stable isotope delta values from experiment six. Dashed line indicates the line of 1:1 agreement.

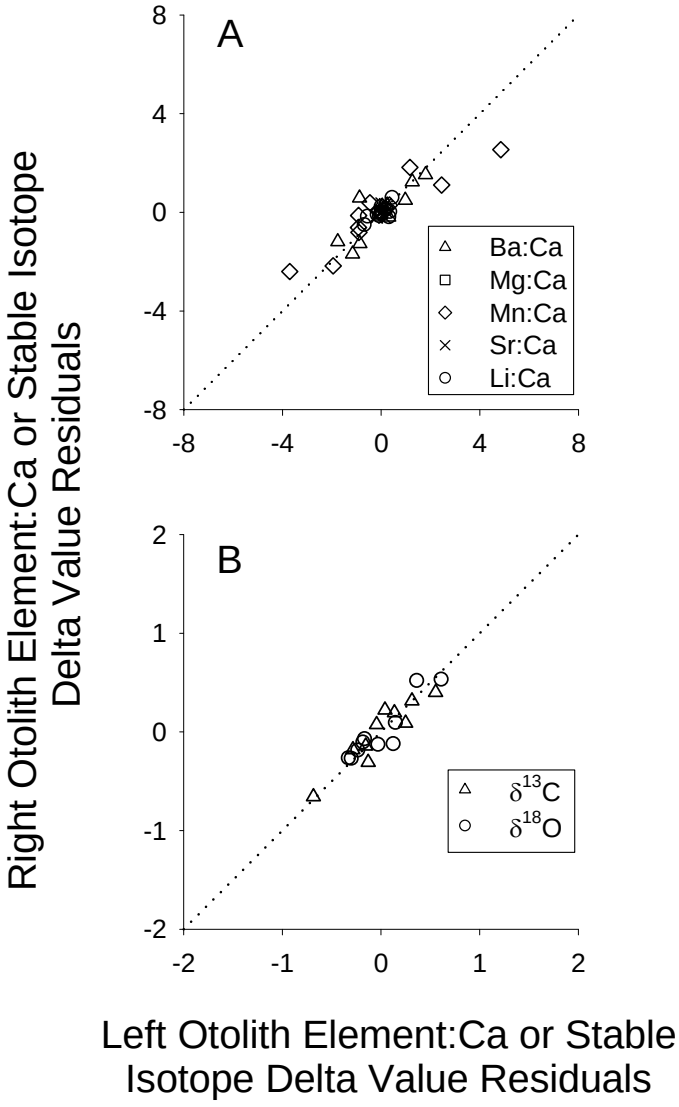

Figure 5. Maps of age-0 red snapper sampling locations in fall 2005, 2006, and 2007

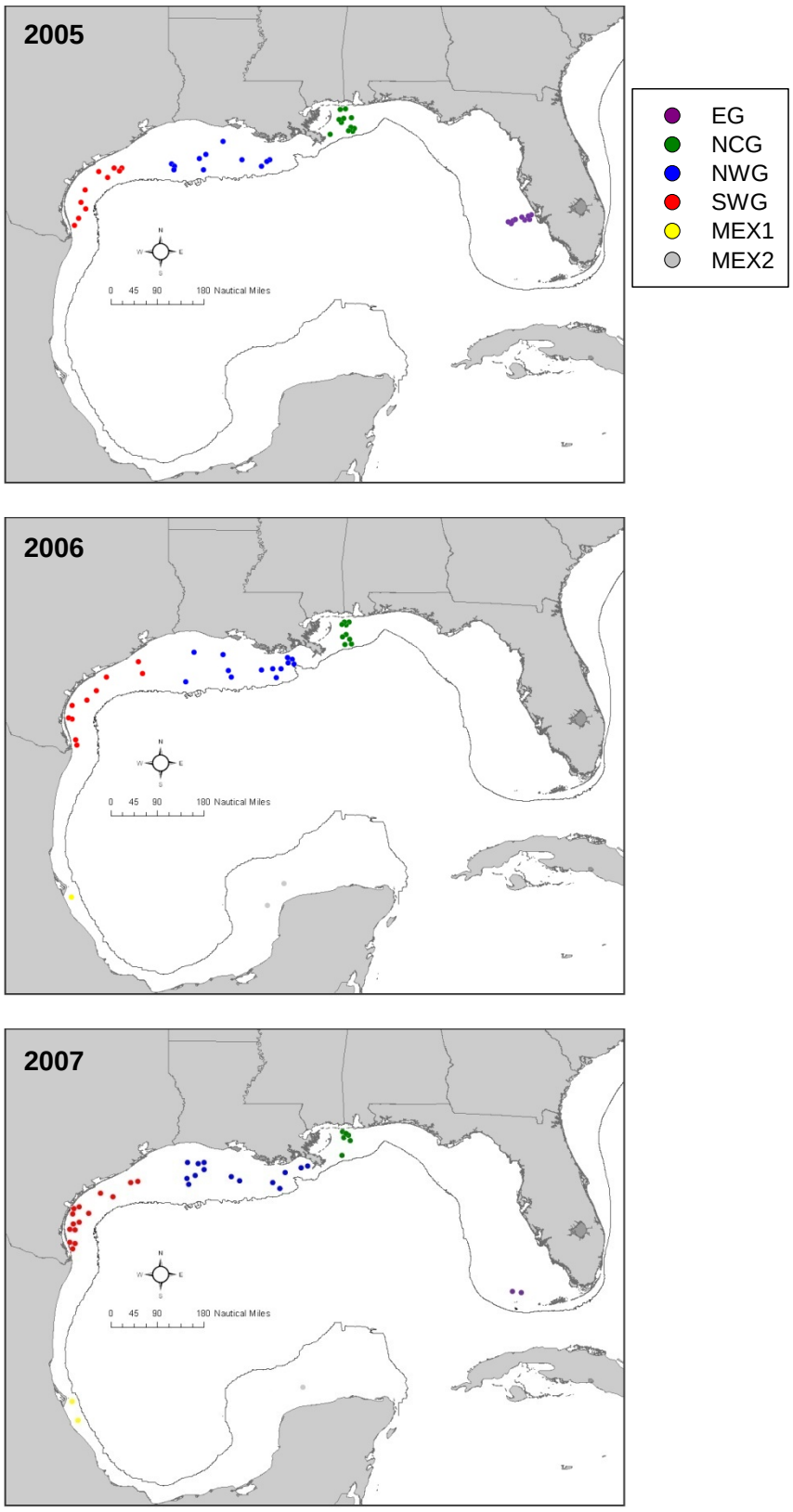

Figure 6. Mean standard length (± SE) of age-0 red snapper sampled in 6 regions of the Gulf of Mexico between 2005 and 2007.

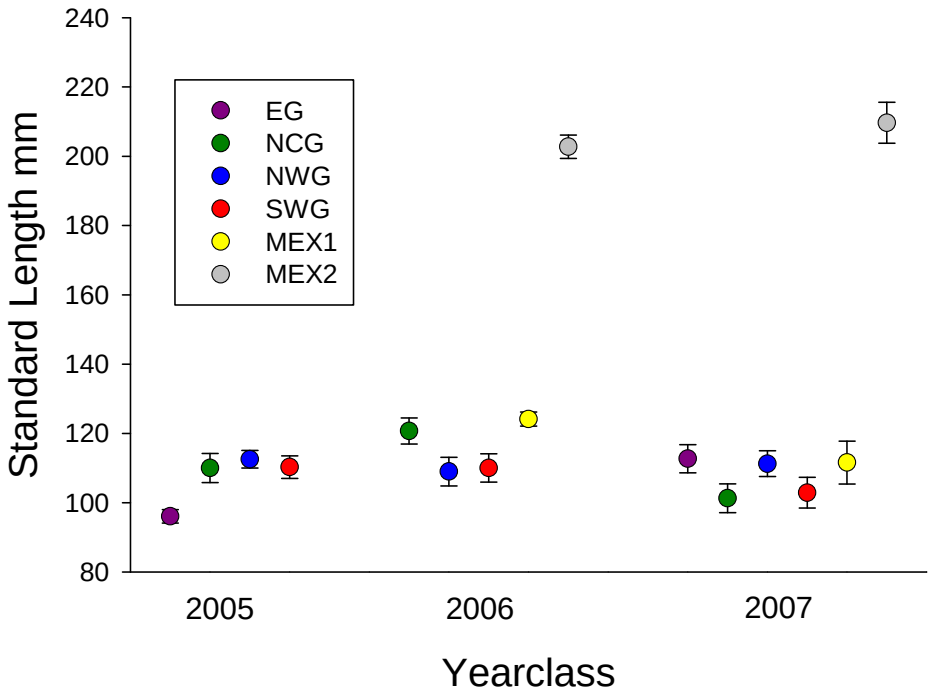

Figure 7. Mean (±SE) region- and year class-specific otolith element:Ca ratio or stable isotope delta values for age-0 red snapper sampled from six Gulf of Mexico regions in fall 2005-2007. Units for each panel are provided in its title.

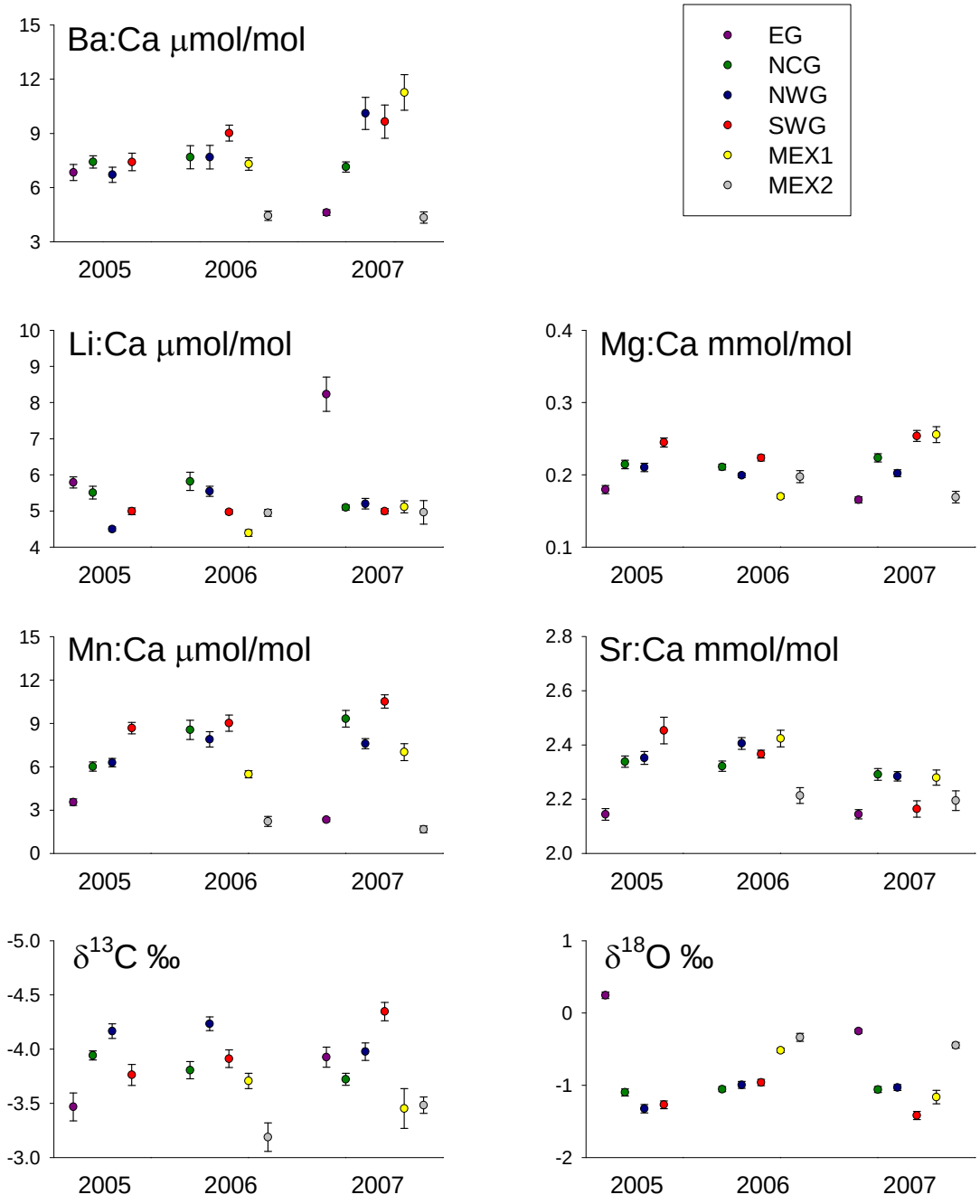

Figure 8. Standard length distributions of red and lane snappers sampled in 4 regions of the northern Gulf of Mexico in fall 2005.

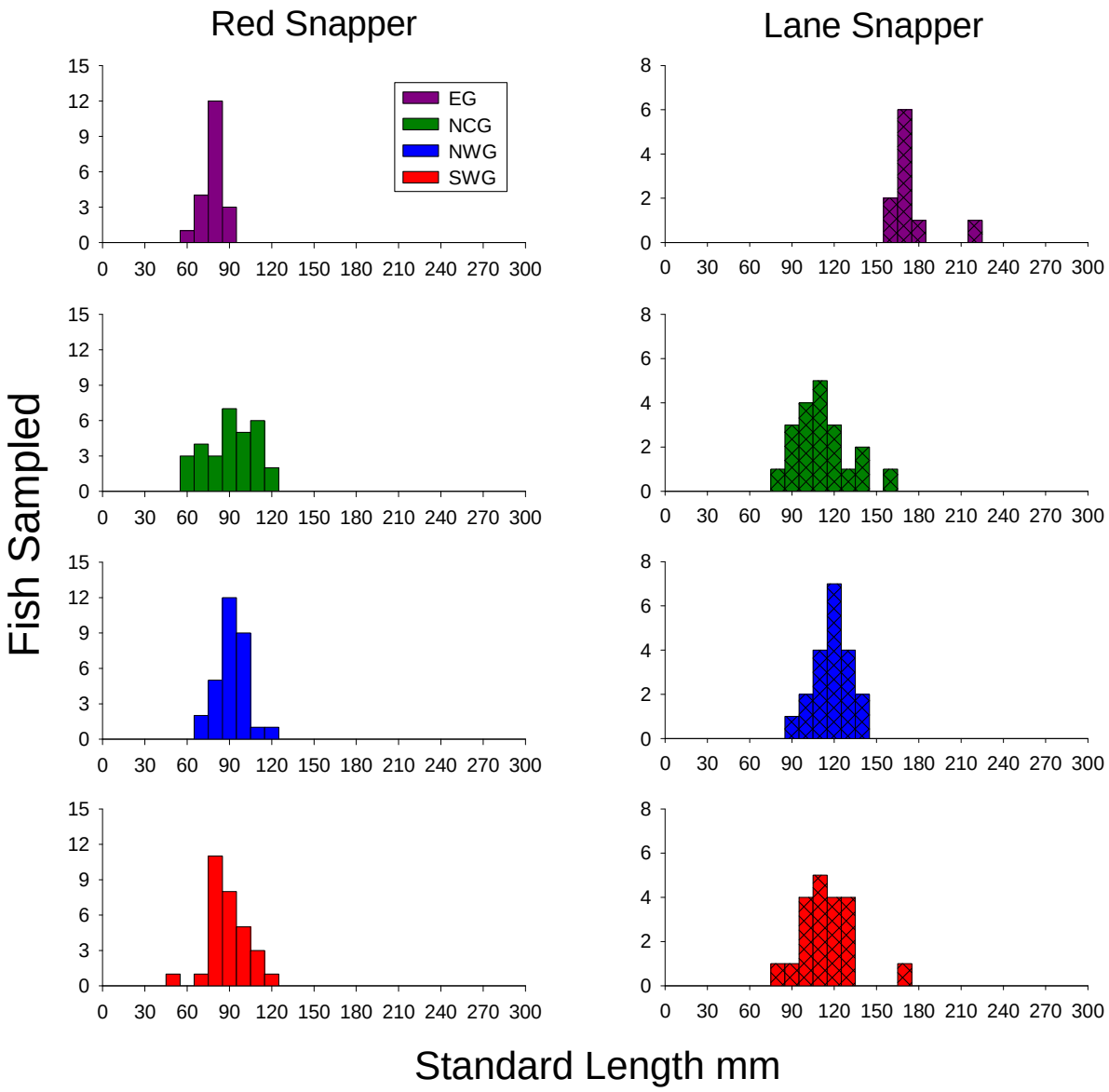

Figure 9. Mean (±SE) region-specific otolith element:Ca ratio or stable isotope delta values for age-0 lane and red snappers sampled from four Gulf of Mexico regions in fall 2005. Units for each panel are provided in its title.

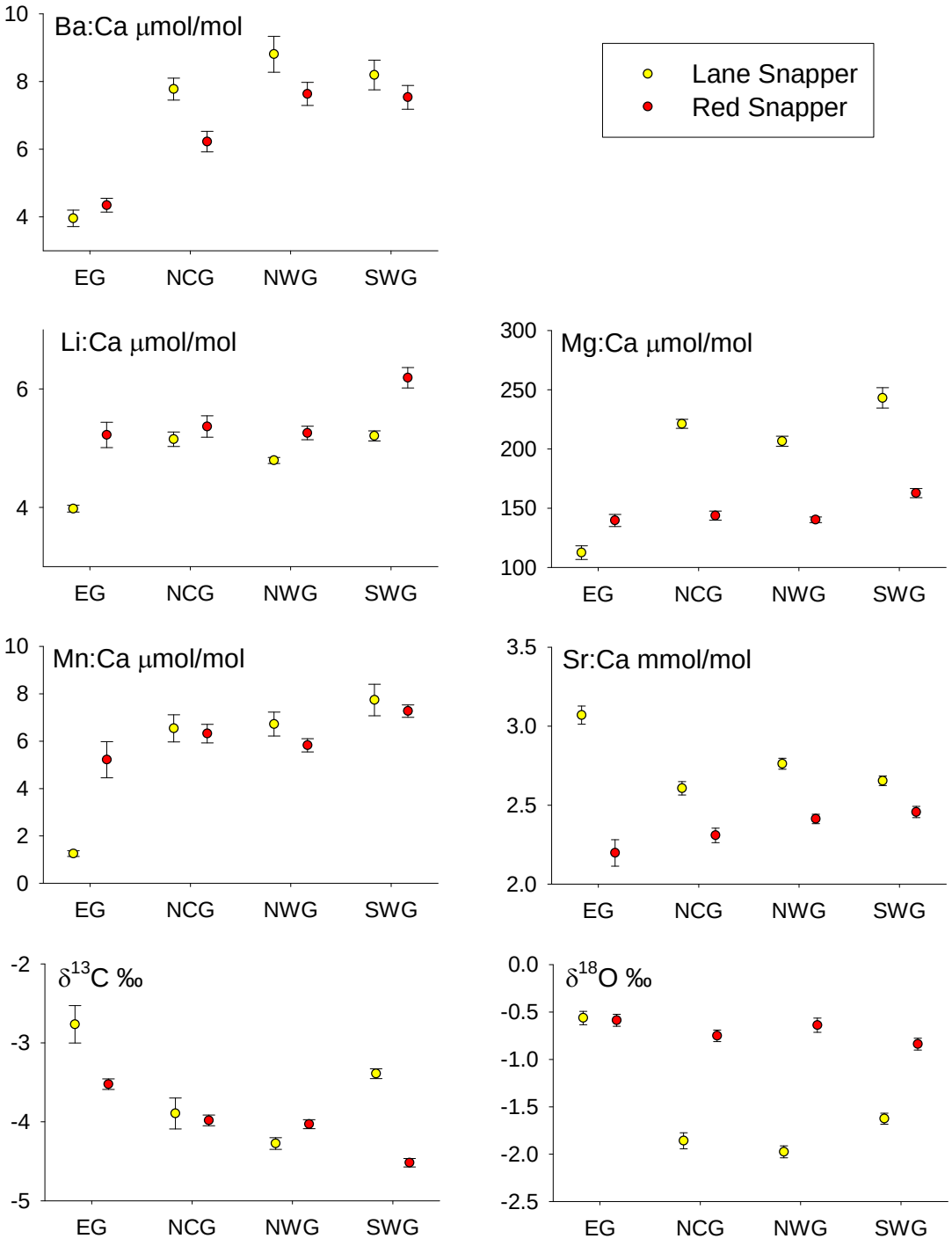

Figure 10. Bar charts indicating percentages of age-0 red snapper sampled from Gulf of Mexico regions in A) 2005, B) 2006, C) 2007, and D) all years combined that were classified to sampling regions with linear discriminant function analysis of otolith chemical signatures.

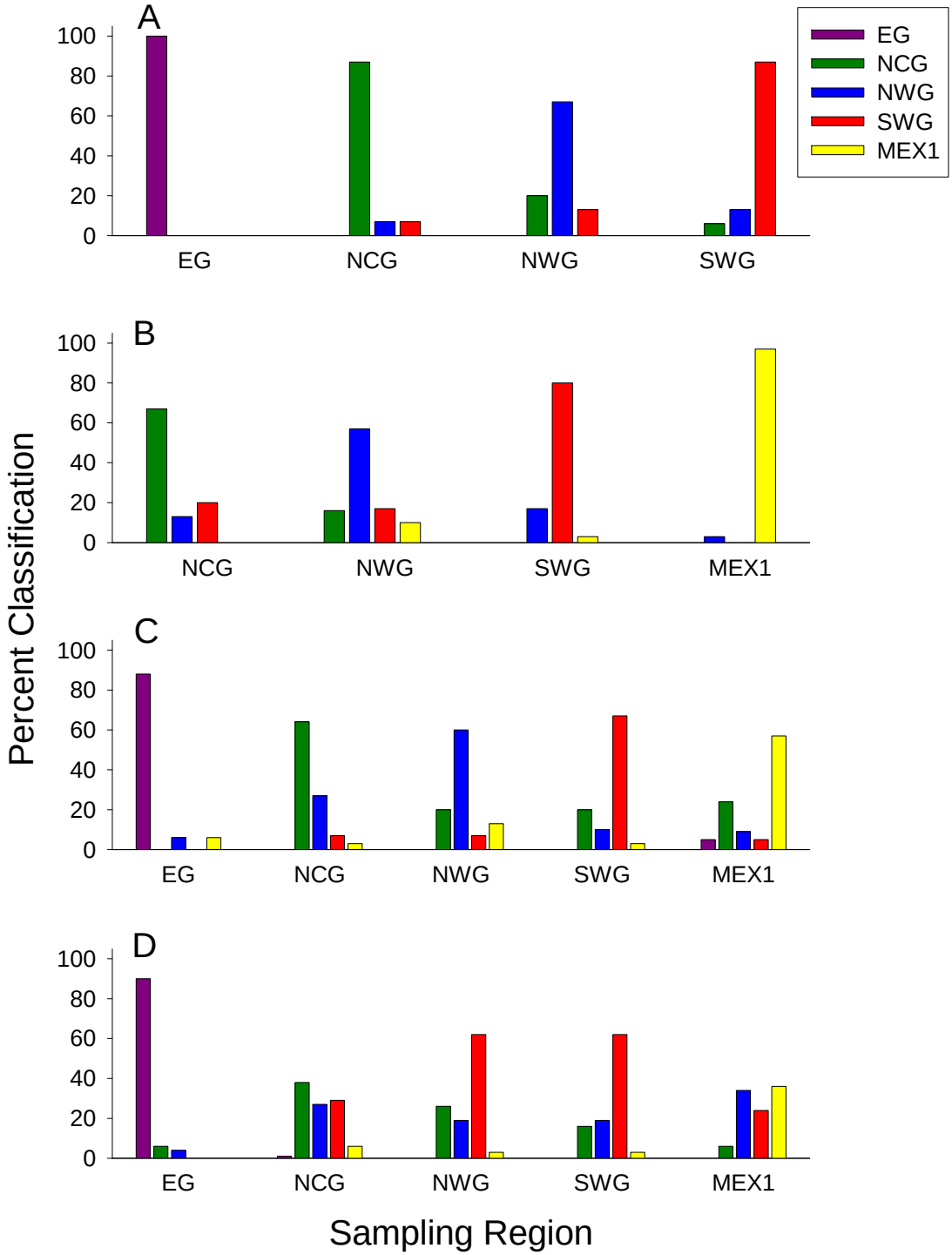

Figure 11. Mean (±SE) region- and age-specific otolith core element:Ca ratio or stable isotope delta values for sub-adult and adult red snapper sampled from six Gulf of Mexico regions in summer 2006-2008 (sampling year). Panel labels indicate the yearclass presented.

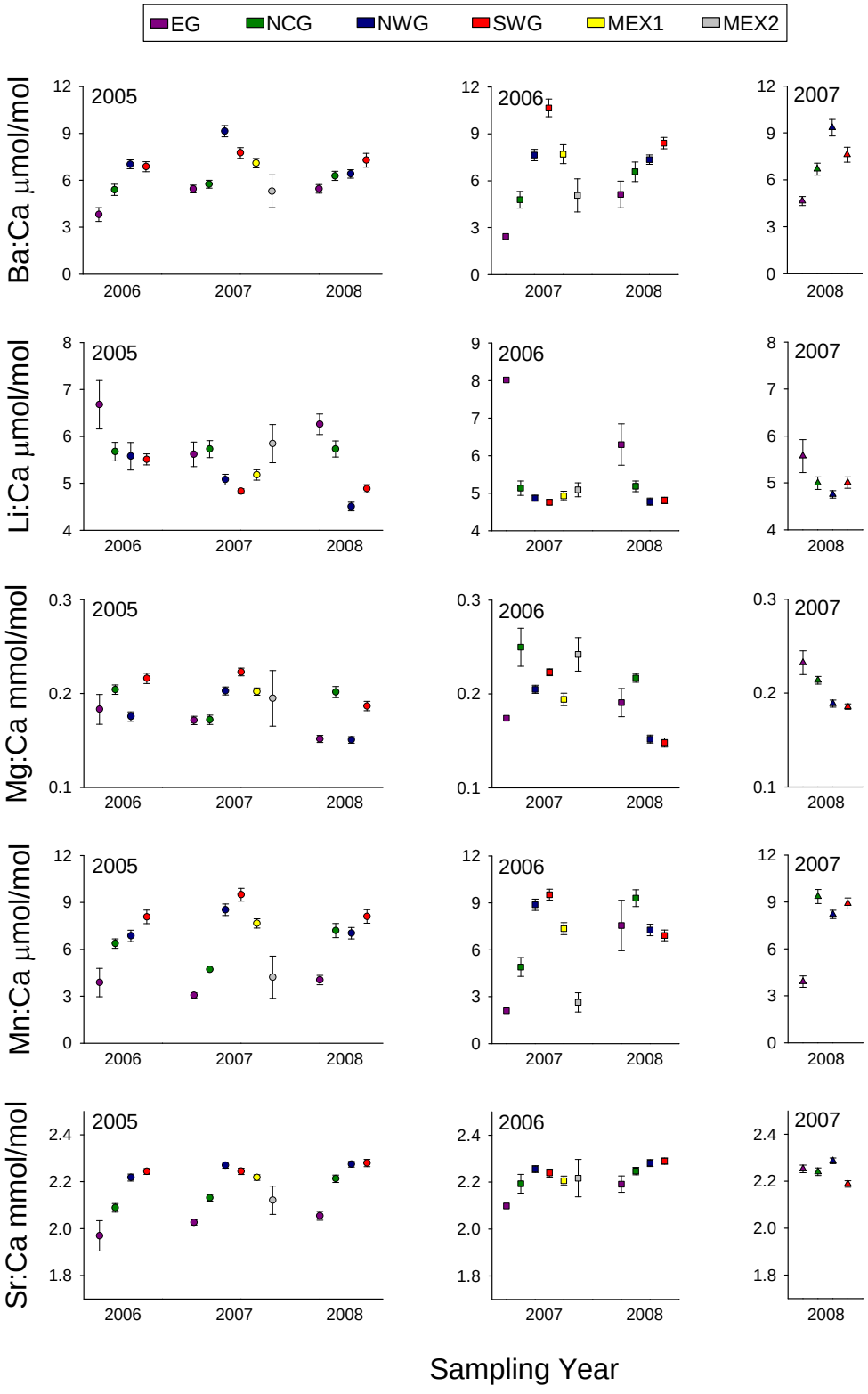

Figure 11. continued.

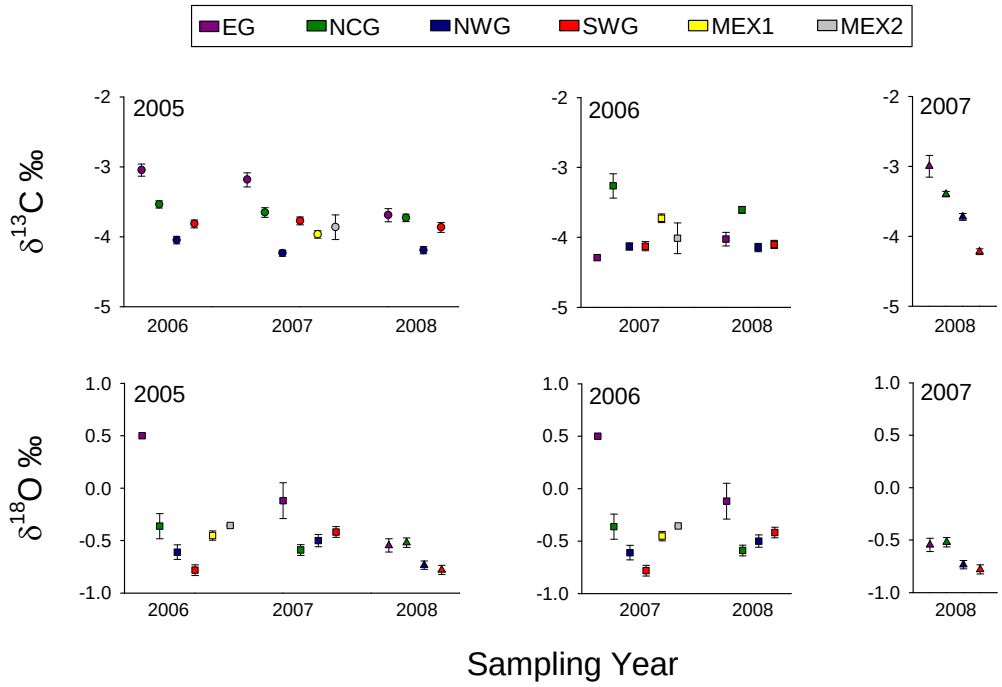

Figure 12. Bubbleplots of otolith chemistry-based maximum likelihood estimates of the percentage of sub-adult or adult red snapper that recruited from regions in which age-0 fish originally were sampled. Sub-adult and adult fish were sampled in each northern Gulf of Mexico region between summer 2006 and summer 2008. Columns are cohort-specific and panels indicate fish age.